Deliverable 3.4
Report on breeding gaps and key factors for strengthening small breeding initiatives:
Experiences on five crops

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

1. Summary

Organic farming is an increasing sector in the agriculture of the world and the European Union. However, most organic production in Europe is based on the seeds from conventionally bred varieties or even on conventional non-organic seeds, while, by contrast, there is a remarkable lack of organic seeds and/or varieties specifically adapted to low-input and organic farming in Europe. The overall goal of LIVESEED Task 3.4. is to support small existing breeding initiatives and initiate new collaborations to close gaps in various crops. The main objectives of Task 3.4. are to determine the most important gaps and key factors which are limiting the availability of varieties adapted to low input/organic farming, to propose solutions and alternatives, to exchange knowledge among already existing (or created ad hoc) organic breeding networks, to develop, reinforce or give support to small organic breeding initiatives in Europe. This was addressed in five case studies of small scale breeding initiatives on five main crops white lupin, Brassica vegetables, apple, wheat and tomato.

Whire lupin is a very interesting crop for organic farming as it is a high-quality food (protein content, digestibility, high quality oil and disease), but also as a legume it contributes to improve soil fertility and to mitigate climate change. However, the available of adapted cultivars is very scarce. In this crop LIVESEED has contribute to widen the genetic base of this crop, working with a plethora of lupin accessions, which have been evaluated and selected under stress and low input conditions (anthracnose, calcareus soils, drought, low winter temperatures) and quality traits, as well as supported by molecular techniques (GBS genotyping). All activities have been developed by means of a network of LIVESEED partners and other European centres, following a participatory approach.

Brassica vegetables are one of the most relevant European crops, which are grown mainly in winter season in Southern countries and in spring/summer season in central and northern Europe. Thus, countries like France, Italy, Portugal and Spain are the main producers for European markets in winter and spring. However, hybrids from cytoplasm male sterile lines (CMS) originated from cell fusion are predominant, which is a paramount concern for organic markets. In this subtask a group of LIVESEED partners and other stakeholders have created a network aimed to perform and coordinate participatory organic trials with cell fusion free varieties and lines of cabbage, cauliflower, broccoli and kohlrabi in the mentioned countries of southern EU to identify alternative varieties and to foster transnational collaboration in this frame. However, in case of broccoli and cauliflower open pollinated varieties from Central Europe were not adapted to winter planting. Therefore, specific breeding programs for Southern Europe are necessary.

Apple is the most important fruit tree in Europe, but their organic production is still based on modern varieties, which have been bred conventionally for high input intensive growing conditions. Thus, there is a need of plant materials (both cultivars and rootstocks) specifically bred and adapted to organic production without copper treatments. Promising candidate cultivars derived from organic apple breeding have been tested across a newly established network of organic farms in Central Europe and degustation of first fruits results were quite promising. A lot of energy was spent to create a forum of discussion to identify actors involved in organic apple breeding and testing across Europe, coordinate the exchange of ideas and materials and to plan coordinated trials in apple organic breeding. The main aim of this subtask was to develop a European network of agents involved in the different points of apple organic breeding (e.g. research centres, universities, breeders, nurseries, retailers).

Winter wheat is a staple crop in the world and particularly in Europe, used in a range of foods and culinary applications. Common bunt, caused by Tilletia caries, is a seed borne disease, which mainly affects the organic sector as it goes from one year to the next when seeds are not treated and, therefore, strongly affects the organic production sector. Furthermore, there is a bottleneck in the current genetic pool in terms of resistance to this disease as this was not addressed in conventional breeding. In this natwork, farmers, breeders and scientists are working in a participatory fashion with a wide collection of varieties and breeding lines, which encompass several sources of resistance genes

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against a broad range virulence strains of T. caries, aimed to breed for resistance to this disease as well as to find molecular markers and QTLs linked to these resistances.
Finally, tomato is the most important fruit vegetable in Europe and, particularly Italy and Spain are the main producers. However, the use of organic seeds is very scarce in these countries, although many small organic farmers and initiatives offer the opportunity of using a higher varietal diversity based on organic seeds and local landraces and ecotypes. In this subtask several small initiatives for organic breeding of tomato have been supported by LIVESEED partners in participatory breeding for local adaptation using collections of landraces from genebanks, composite cross populations from previous EU projects or MAGIC experimental populations.
All the initiatives mentioned have contributed, according to the characteristics of each crop, to fill the gaps identified at the beginning. Activities, preliminary achievements and challenges of each crop specific network were presented. Trustful relationships were established among small breeding initiatives that strengthened the network and collaboration which should help to tackle identified new breeding gaps beyond LIVESEED project work. Each network reached out to other actors and stakeholders and also to the partners of the sister projects ECOBREED and BRESOV. Lessons learned from the five case studies allowed to identify key factors for successful collaboration to support or initiate new small organic breeding initiatives in different crops and regions of Europe. Besides crop specific issue the overall framework with respect to science and technology, market, society, norms and regulations need to be considered. An engaged facilitator is key for successful collaboration but also involvement of the whole value chain (farmer, processor, trader, consumer) to meet the market demand. Policy makers and society need to be addressed to create awareness for the added value of organic farming and plant breeding in respect to sustainable food production following the 'farm to fork' strategy of the EU commission. The perspective of long-term funding is most decisive for (i) the future of existing organic breeding initiatives to attract young breeders, (ii) entrepreneurs who want to start new organic breeding programs, and (iii) for conventional seed companies to invest in organic seed and breeding.

2. Introduction

LIVESEED is based on the concept that cultivars adapted to the unique conditions inherent in organic systems are the key for eco-functional intensification and realization of the full potential of organic agriculture in Europe. Innovations facilitating breeding, registration, multiplication and a wide use of such cultivars will ensure sustainable and resilient food production of organic and low-input agricultural systems, thereby, increasing their competitiveness. LIVESEED integrates different disciplines to address the different framework conditions (*Figure 1*) and applies the multi-actor and action research approach ('learning by doing'), based on the close collaboration between project end-users (breeders, seed producers, farming associations, processors, retailers, traders, certification bodies), researchers as well as public authorities in relevant stages of the project. This cooperation is crucial to (i) understand the existing bottlenecks related to the low use of the organic seed and (ii) co-develop solutions adapted to the needs of the project end-users (market-oriented approach). Furthermore, it allows innovation through co-construction of knowledge and knowledge sharing, which is vital to guarantee a broader application and upscaling of organic breeding and seed production. Involvement of LIVESEED partners across Europe enabled the process of common building of knowledge between countries.

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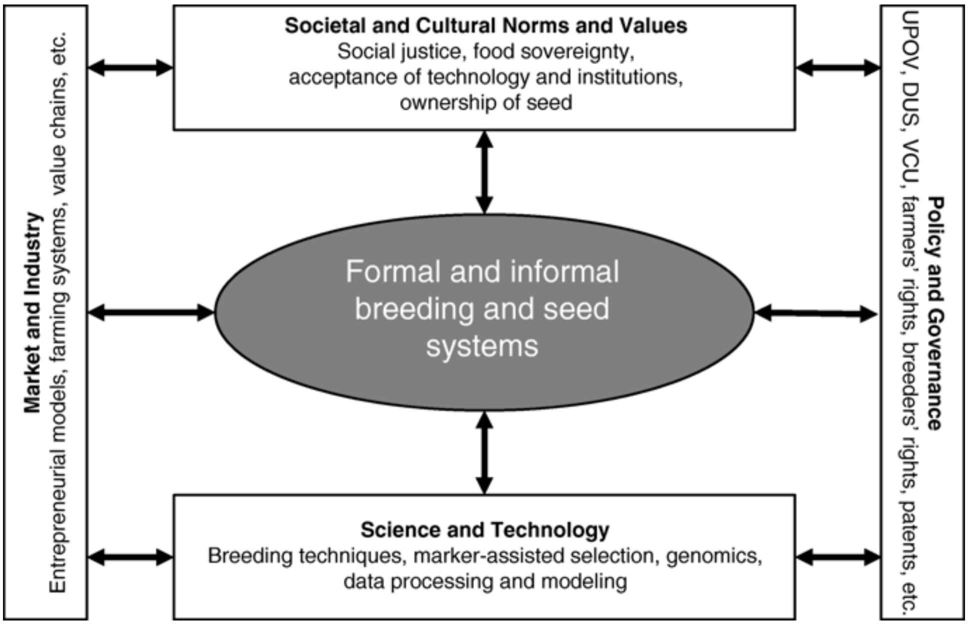

Figure 1 Roles and positioning of the organic breeding and seed systems within their technical, economic, institutional and cultural context derived from Lammerts van Bueren et al. 2018.

There are still many gaps in the choice of suitable cultivars adapted to organic and low-input farming in Europe as most breeding is not considering traits crucial for organic farming systems. Reasons are not only technical but mostly institutional and socio-economic in nature (Van Loqueren and Baret 2008). There are several organic small breeding initiatives aimed to adaptation to local conditions and markets. In addition, both the formal sector (universities, research centers, seed companies) and informal sector (small breeders and farmer-breeders) must still overcome knowledge gaps and build bridges among the actors involved in these activities, in order to achieve a more efficient interconnected organic breeding.

Task 3.4 aimed to work with a number of representative species as case studies for building interconnected organic breeding initiatives and by doing so to have a more comprehensive understanding of socio-cultural, economic and technical factors that are important to mitigate the breeding gaps in these crops and to identify the most important key factors the stimulate the development of new initiatives. The considered species are:

- White lupin (3.4.1, lead CREA-IT)
- Brassica vegetables (3.4.2, lead BNN-DE)
- Apple (3.4.3, lead AEG-GR)
- Winter wheat (3.4.4, lead Uni Kassel-DE)
- Tomato (3.4.5, lead UPV-ES)

Each species (sub-task) has different approaches, challenges, breeding objectives, socioeconomic factors and market conditions. The objective of this 3.4 task is to work on already a priori identified gaps and needs, as well as to identify new gaps and challenges. Moreover, the contact, discussion and exchange of knowledge among the actors involved (from farmers to scientists, from retailers to consumers) is essential to (i) clearly identify solutions, (ii) boost the already existing small initiatives and (iii) stimulate further collaborations. Lessons learned in this research are translated into key-factors important to create and encourage new initiatives.

Based on the results of the first 36 months of activities, the objectives of this report are to:

- Determine the most important gaps and key factors, which are currently limiting the availability of cultivars adapted to organic/low input farming specific for each species and common to several or all species.

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- Describe the different approaches to overcome potential limitations in new collaborations for other crops
- Report on preliminary achievements from the activities of each subtask and to check how these activities have contributed to solve gaps and limitations
- Identify generic key factors to foster collaboration and efficiency of organic breeding activities aiming for improved cultivars with adaptation to organic farming and good acceptance (or high potential) for the market and/or consumers.

In the next sections we will provide a brief background and gaps to be filled for each crop (Chapter 2), the main activities during these three years (Chapter 3), preliminary achievements and challenges to close gaps (Chapter 4) and key factors for strengthening small organic breeding initiatives (Chapter 5).

2. Brief background of each subtask and gaps-to-be-filled

2.1. White lupin: Fostering collaboration in breeding and widening the genetic base by composite cross populations (CCPs) and molecular approaches

Lead: CREA (Paolo Annicchiarico)

Project partners: FiBL-CH and GZPK, LBI, UBIOS

Other collaborators/stakeholders: James Hutton Institute (Dundee, UK), Institute of Industrial and Forage Crops(Larissa, Greece), Instituto Nacional de Investigación Agropecuaria (INIA, Carillanca, Chile), Eric von Baer (Chile), AssoSementi (Association of the Italian seed companies, Italy), Jouffray-Drillaud (France), Instytut Genetyki Roślin Polskiej Akademii Nauk (PL), DSV (DE), Bayrische Landesanstalt für Landwirtschaft LfL (DE), Western Australian Agriculture Authority (AU) and genebanks in Germany, Russia, Spain and Australia.

White lupin (*Lupinus albus* L.) offers great potential as a high-quality food (high protein content; high-quality oil; high digestibility; hearth-protecting and anti-diabetes properties) and feed crop. Therefore, it is a very interesting crop to diversify organic production and, as a legume, to improve soil fertility, mitigate climate change and reduce crop energy costs via symbiotic nitrogen fixation.

Main concerns/gaps-to-be-filled that prevent or limit the production of white lupin in organic and conventional agriculture are biotic and abiotic stresses and anti-nutritional compounds like quinolizidine alkaloids responsible for bitter taste.

Tolerance towards anthracnose caused by soil and seed-borne fungi *Colletotrichum lupini*, calcareous soils, winter hardiness and drought tolerance envisaged to be the main stresses to be coped with in this crop. On the other hand, the genetic pool used for decades in breeding has been limited to few sweet-seed (low-alkaloid) genotypes that have been discovered only in 1927. Since then domestication and breeding of white lupin is based on few sweet mutants. Although 9 genes have been identified to influence alkaloid contents mainly one "pauper" locus is used in breeding on global scale (Rychel S. and Książkiewicz M. (2019) causing a genetic bottleneck. Therefore, it is of paramount importance to widen the pre-breeding genetic pool by hybridization with bitter landrace germplasm. This would increase the opportunities of finding genotypes resistant or more resilient to the mentioned stresses, and possibly richer at the nutritional level.

A breeding network (including European and foreign institutions) of initiatives was planned at the beginning of the project. CREA was in charge of broadening the genetic base by developing, multiplying, evaluating, and distributing for further evaluation a number of advanced sweet-seed lines

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obtained from crosses between elite modern low-alkaloid lines and selected bitter landraces identified from prior multi-environment studies in Europe.

A population of about 600 advanced lines has been developed after preliminary selection for low alkaloid content, with on-going testing of a subset of about 150 genotypes for tolerance to calcareous soils (LBI), severe drought (CREA), low winter temperatures (planned for UBIOS and later on performed to some extent by CREA), and grain quality based on low alkaloid content, high oil content, and high protein content (CREA). Testing for tolerance to anthracnose was planned for the same 150 genotypes by FiBL-CH if sufficient variation for anthracnose tolerance was detected among the parent material. As an alternative a large number of genebank accessions, and own breeding lines and released cultivars will be screened by FiBL-CH for anthracnose tolerance using field trials. The development of a screening tool under controlled conditions allowed the phenotyping of 200 white lupin lines in order to identify molecular markers for anthracnose tolerance.

In addition, evaluation of the generated lines in other regions and environments is on-going in Scotland, Greece and Chile (performed by non-partner institutions using own financial resources).

Presently, the set of 150 test lines and a large set of other lines including germplasm of FiBL-CH are undergoing genotyping-by-sequencing characterization, in order to jointly analyse phenotypic and genotypic traits to build up genomic selection models and identifying key molecular markers that could be used to screen the remaining, non-tested lines and/or other germplasm collections for stress tolerance or grain quality traits listed above. Also, the set of 150 test lines is being characterized by NIRS, to define NIRS-based selection tools for grain quality.

The initial plan to develop a CCP population (as in other inbred species) was modified, because of the overwhelming need to select for low alkaloid content – a step necessarily performed on inbred lines – to limit the possibility of quick evolution towards high alkaloid content of a population. Hence, populations may only assume the genetic structure of mixtures of elite inbred lines in this species. This activity will lead to distribution to project partners of an evolutionary population (useful for breeding and/or further selection under specific stress pressures) by the end of the project.

2.2 Brassica: Initiate a European network for cell-fusion free Brassica vegetables

Lead: BNN (1-K. Arp, 2-T. Kimmel, 3-J. Zellfeder, 4-Holger Scharpenberg)
Project partners: SATIVA, Kultursaat, BSG, LSSV, ITAB, FiBL-CH, INRAe, UPV, Vitalis, ECO-PB
Other collaborators/stakeholders: Bejo P.A.I.S. Plateforme Agrobiologique d´Experimentation de Suscinio (France), LA UNIÓ de Llauradors, Agrologic Val, La Verde Coop., SURINVER farmers Association (Spain), Organic Seed Alliance (USA)

Brassica crops are highly appreciated vegetables for their taste and nutritional properties. In Northern Europe they are generally cultivated from spring till autumn, while in Southern EU countries they are particularly cultivated during the winter season, where they are mainly produced for the Northern European market. These crops are therefore of paramount importance for the organic sector.

Main concerns/gaps-to-be-filled is the need for a wide choice of the large range of Brassica vegetables that are derived from breeding techniques which are in compliance with the organic sector and show high performance and high quality which is accepted by the market.

Consumers of organic products are highly concerned about cell fusion-based cultivars in Brassica as they are developed with biotechnological strategies. Nowadays cytoplasmatic male sterility (CMS)

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derived from cell fusion is commonly used in the development of F1 hybrid cultivars. Therefore, there is an urgent need to replace these cultivars by others coming from other (organic friendly) breeding strategies, e.g. F1 hybrids using self-incompatible parent lines or open pollinated varieties (OP), evaluate their performance under organic cultivation in different climatic regions in a multiactor approach and to forster organic breeding initiatives. Traders in Central Europe have a strong demand for produce from cell-fusion free varieties, but they rely during certain seasons on produce coming from Southern countries like Spain or Italy. Therefore, it is important that cultivar alternatives are not only available for Central European conditions, but also for Southern Europe.
To achieve that a participatory plant breeding (PPB) network consisting of a range of organizations has joined forces:

- Organic breeding initiatives: Kultursaat, Research institutions: LBI, INRAe, ITAB, UPV, FiBL-CH
- Small organic breeding and seed companies: SATIVA, LSSV, BGS
- Commercial breeding companies: Bejo, Vitalis
- Organic trading organization: BNN

In order to:

- Coordinate and support trials and new organic breeding initiatives in Southern European countries (France, Italy, Portugal, Spain), based on cell fusion free genetic resources and fitted to the approach of BNN as wholesaler.
- Identify the best adapted cell fusion-free cultivars to organic cultivation under organic cultivation in Central and in Southern EU countries

ECO-PB is also involved in the networking and promotion of these cell fusion free cultivars for organic agriculture and the political lobbying for transparency of breeding techniques of released cultivars.

2.3. Initiate European organic apple/rootstocks breeder network

Lead: AEG (Kostas Koutis)
Project partners: ITAB (FR), GRAB (FR), FiBL-CH (CH), POMA CULTA (CH)
New members/collaborators/stakeholders: IPC (PT), SEAE (ES), CRAW-W (BE), AGROSCOPE (CH), SERIDA (ES), BIOFRU (GR), Farm Hellas Nursery (GR), AUTH (GR), Pomology Institute-HAO DEMETER (GR), Copenhagen University (DK), APFEL:GUT (DE), KOB (DE), LWVO (DE), University Hohenheim (DE), University Oldenburg (DE), State Research Institute for Pomiculture (DE), Gavle University (SE), Holovousy Institute of Pomology (CZ), Vienna University for Natural Resources (AT), VZ Lainburg (IT), Ministry of Agriculture (SRB), Luke Natural Resources Institute (FI), Fruit Growing Institute (BG), , CRPV (IT), AgroBio47(FR), UASVM (RO)

As the most relevant fruit tree in Europe, apple is facing many challenges in the sector of sustainable and organic production. Most cultivars can only be produced economically with copper application to combat fungal diseases. Due to its environmental side effects, copper will be banned in the near future and need to be replaced by more holistic plant protection strategies. Breeding for more robustness is one of the most sustainable approaches, but is still in its infancy in Europe as it requires more than 20 years to breed and introduce new apple cultivars. Pioneers like Poma Culta have already started decades ago with organic apple breeding, however, their progress is hampered by limited resources for breeding, testing and marketing of new candidates.
Main concerns/gaps-to-be-filled is the need of genotypes more resilient and adapted to organic agriculture and soil fertility conditions and new pests and diseases. This includes both cultivars (scion) but also rootstocks.
The main aim of this task is to develop an EU network which joins already existing initiatives in organic apple breeding and to include new ones as a way of establishing a common working group to:

- Link actors across Europe involved in organic apple (fruit) breeding
- test new candidate genotypes under organic conditions in different countries
- share knowledge and genetic resources to enhance organic apple and fruit breeding.

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Poma Culta runs apple breeding under biodynamic conditions and has established a first network to test the 5 most promising apple genotypes in Switzerland and neighboring countries. Complementary resistance screening as well as sensory testing are conducted by FiBL-CH in close cooperation with Poma Culta. The application of modern breeding tools like molecular makers that have already been published (in former projects like FRUITBREEDOMICS in EU or RosBREED in the US) were evaluated and implemented in the apple breeding of Poma Culta.

2.4. Winter wheat: Supporting organic winter wheat breeders by providing modern breeding tools to improve resistance to seed borne diseases: the case of *Tilletia caries*

Lead: UNI KASSEL (Gunter Bakes, Jelena Baćanović-Šišić)
Project partners: AGROL, Cultivari and ZF Dottenfelderhof organic wheat breeders
Other collaborators/stakeholders: ECOBREED: BOKU (AT)

Wheat is a staple crop and the most important cereal in Europe as it is used to make a plethora of foods, beverages and other food products (e.g. bread, pasta, beer) and feed for cattle, chicken, etc. However, there is a serious problem with seed borne diseases like common bunt caused by fungi *Tilletia caries* in organic wheat as synthetic seed treatment is not allowed. With expansion of organic agriculture in Europe bunt diseases are on rise. This also affects organic farmers growing own selections, cultivar mixtures, conservation varieties and populations. Since in conventional wheat production the seed is regularly treated, resistance breeding for seed and soil born diseases has been of minor importance.

Main concerns/gaps-to-be-filled is to develop wheat cultivars, mixtures and populations with improved tolerance towards seed and soil born diseases to secure organic production and reduce input of seed treatments in low input production.

LIVESEED will focus the breeding research on common bunt and the use of modern breeding tools to broaden the resistance of organic wheat to *T. caries*. The starting point was about 450 winter wheat lines: i) 420 F8 lines derived from composite crosses of a diverse/exotic lines with different new and effective resistance genes against common bunt developed in Denmark by AGROL and ii) 30 lines from the German organic breeders Cultivari and ZF Dottenfelderhof with bunt breeding programs as sources of new resistances to *T. caries*. These lines were chosen in order to encompass seven resistance genes (Bt1, Bt2, Bt5, Bt7, Bt13, BtZ and Quebon resistance), distributed more or less equally among them.

The task involves different working lines:

- AGROL: To phenotype on-the-field the resistance of 300 breeding lines to 10 highly virulent *T. caries* races (2 years)
- UNI KASSEL: To genotype these breeding lines by Genotype by Sequencing (GBS)
- UNI KASSEL: To perform a genome wide association study (GWAS) between resistance phenotyping and genotyping, searching for genetic markers associated to resistances
- To test the most promising lines in the facilities of CULTIVARI and ZF Dottenfelderhof, including agronomic behavior and baking quality

2.5. Tomato: Support participatory tomato breeding in Italy and Spain

Lead: UPV (Adrián Rodríguez-Burruezo)
Project partners: RSR, Arcoiris, CREA,
Other stakeholders/collaborators: ITALY: Organic farms for PPB trials Azienda Agricola Celestino Benetazzo (Padova, PD), Azienda Agricola Malavolta, (Campofilone, FM), Azienda Agricola Corrado Salvatore (Metaponto, MT), Associazione Diversamente Bio (Rubano, PD), Azienda Agricola Alle

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Fontanine (Sestola, MO), Azienda Agricola Di Leo Silvano (Castronuovo S.A., PZ), Azienda Agricola Battezzato Vincenzo (Campobasso, CB), Azienda Agricola Petacciato (San Giuliano di Puglia, CB), Azienda Agricola Primo Sole (Montagano, CB), Azienda Agricola Sperimentale Didattica Pollino (ALSIA Rotonda, PZ); SPAIN: La Verde Coop. (Cadiz, Andalusia) and LA UNIÓ de Llauradors (Valencia) supporting main field trials and PPB in Spain.

Tomato is the most important fruit vegetable in Europe and Italy and Spain are the main producers of organic tomatoes in the EU.
Main concerns/gaps-to-be-filled is a lack of available organic seed and, apart from small initiatives, most varieties are modern F1, which were bred under high input agriculture, with a considerable lack of diversity and fruit quality and taste and specific adaptation to organic systems. Thus, on the one hand there is a need of high-quality cultivars adapted specifically to organic production and stress conditions and, on the other hand, small initiatives aimed to preserve, recover and improve ancient landraces must be supported.
The tomato task is an ambitious project which was planned to work with different populations and approaches, having in common organic conditions and participatory evaluation of populations for local conditions. Thus, in Spain UPV team have been evaluating a collection of about 250 landraces and local varieties in two main locations of Spain, with different agroclimatic conditions (Valencia and Villamartín-Cádiz). In Italy: i) advanced lines from a MAGIC population, (based on 8 divergent parents and encompassing a range of fruit qualities, salt and cold tolerance, resistances to fungal and virus diseases, etc.) and ii) Composite Cross Populations "Cuore di Bue" tomato (i.e. ox heart) are being evaluated and selected by farmers under organic condition in four different locations.
The task involves:

- To evaluate a large and diverse collection of ancient landraces in Valencia and Andalusia in three seasons, based on participatory plant breeding (PPB) for agronomic value (e.g. yield, management, farmers´ preferences, incidence of diseases) and fruit quality (mainly sugars and acids)
- Each year, in the second half of trials, to perform shelf life and taste evaluations with consumers and retailers on a pre-selection of landraces
- In Italy to evaluate under organic conditions in a participatory approach a collection of advanced lines from an experimental MAGIC population so that these breeding lines are being selected during their breeding and gene fixation process for organic conditions as well as the traits of interest (agronomic value, fruit quality, resistances, etc.) (CREA)
- to evaluate and select in PPB approach an experimental composite cross population (CCP) of traditional tomatoes, i.e. a "Cuore di Bue". This CCP was created crossing four local varieties selected by farmers during the SOLIBAM EU FP7 project (RSR and Arcoiris)
- To develop, select and release for the organic sector seeds of improved and competitive varieties from the genetic pool of ancient landraces, contributing to preserve agrobiodiversity and to improve fruit quality for consumers.
- To genotype by DNA fingerprinting the best cultivars derived from the MAGIC population and to exploit heterosis by planning crossings between genetically diverse parents.

3. Description of activities on each subtask/species

3.1. White Lupin

In the first year, CREA generated a genetic base of 580 white lupin inbred lines originated from paired crosses between four elite, bitter-seed landrace parents and four elite, sweet-seed breeding lines/varieties, with preliminary selection for low alkaloid contents performed on single seeds by the

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fluorescence test and by a new, possibly more sensitive experimental procedure that still requires thorough validation.

A subset of 190 inbred lines were multiplied and preliminarily evaluated for flowering time and grain yield under spring sowing in Lodi, using a randomized complete block design with three replications. This experiment, which included also 12 genotypes from LBI, served as well to collect seed samples for future grain quality evaluations. Preliminary research work started as well at LBI (multiplication of own breeding lines). In the second year, a core set of 144 lines were evaluated for drought tolerance by CREA in a replicated experiment performed in large phenotyping platforms, assessing the material under severe drought and moisture-favourable conditions (*Figure 2*). The same core set was evaluated by LBI for adaptation to moderately high soil lime and pH, but plant emergence was severely hindered by outstandingly unfavourable climatic conditions. The resowing of some spare seed allowed only for the establishment of one replicate. In addition, lime soil damage on the plants seemed modest, probably because of severe summer drought which limited the release of calcium in the soil and the plant growth.

Contacts with UBIOS clarified that this partner would not be able to test the core set of 144 lines for cold tolerance. Therefore, CREA implemented an additional, unforeseen multiplication and evaluation under autumn sowing of the same set of lines that underwent spring-sowing evaluation, using an RCB design with two replications. Unfortunately, the relatively mild winter did not allow for a thorough assessment of the cold tolerance trait, owing to modest winter plant mortality. However, this experiment provided a second, contrasting environment for evaluation of grain quality traits.

The partner UBIOS implemented the farmer-participatory evaluation of about 30 breeding lines provided by Jouffray-Drillaud and CREA under autumn sowing in France. This evaluation was performed during the first project year, which implied a fairly mild winter, and continued in a following year with a subset of well-performing lines.

FiBL-CH has developed a screening tool for anthracnose tolerance of lupins using artificial infection of *Colletotrichum lupini* under controlled conditions and a methodology for anthracnose tolerance assessment under natural infection pressure in the field. In Switzerland anthracnose is the most severe disease in lupin, while this disease does not appear in Italy or the Netherlands. The screening tool under controlled conditions was validated with adult resistance in replicated field trials at two sites in 2018 and 2019 by FiBL-CH and GZPK. FiBL-CH tested the 8 parental lines of the CREA population which was provided by CREA in 2018 and found very little variation and high susceptibility against anthracnose for all lines. Therefore, it was decided by the project team that FiBL-CH will not assess tolerance to anthracnose of the set of 144 genotypes as chances are very low to recover anthracnose tolerant genotypes. Instead, FiBL devised alternative collaborative work on this trait with CREA, namely the evaluation of a world collection of 160 landraces and 40 breeding lines and cultivars selected for anthracnose tolerance by FiBL-CH. The landraces were genotyped already by CREA in an earlier project, FiBL is genotyping the remaining 40 lines on its own costs. FiBL-CH has phenotyped these 200 lines in 2020 for anthracnose resistance with the validated screening tool. In addition most promising lines for previous screens are presently tested in in field trials with natural inoculation at two sites in Switzerland. This will be the basis for genome wide association studies to identify markers linked to anthracnose tolerance.

Contacts were taken, and agreement were set, for the phenotyping of the core set of 144 lines for grain yield in other regions and environments. The scheduled work implied evaluations; (i) in Scotland under autumn sowing in 2019 and spring sowing in 2020, by the James Hutton Institute (a Scottish Charity research institution); (ii) in Greece under moderately high soil lime content and autumn sowing in 2019, by the Institute of Industrial and Forage Crops of Larissa; (iii) in the Araucanìa region of Chile under autumn sowing in 2020 by INIA Carillanca (near Temuco). All these evaluations are being funded by own financial resources of these institutions. The work in Scotland will provide, inter alia, a second assessment of cold tolerance; that in Greece, a second assessment of lime tolerance under climatic conditions that contrast with those in the Netherlands; and that in Chile, an additional evaluation under cool Mediterranean-climate conditions.

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Genotyping work has regarded so far the genotyping-by-sequencing characterization of 376 genotypes, including 12 genotypes from LBI and 364 genotypes belonging to CREA's sweet-seed genetic base (including all of the genotypes that underwent some phenotyping work). Additional genotypes belonging to CREA, FiBL and INIA Temuco are going to be genotyped. Preliminary results revealed the availability of several thousands of polymorphic SNP markers, while brighter opportunities in terms of number and position of SNP markers are offered by the definite SNP calling that will be performed using the white lupin genome sequence that has just been published (Hufnagel et al. 2020) and made freely available in France https://www.whitelupin.fr/.
FiBL-CH and GZPK conducted several farmers field days to promote lupin as new or reintroduced crop in Switzerland. Results were presented in farmers newspapers, multimedia and in several scientific conferences.
On-going work includes:

- the second year of evaluation for adaptation to moderately high soil lime and pH, by LBI (successfully established)
- evaluation of grain quality traits, by CREA
- evaluation of a world landrace collection for tolerance to anthracnose by FiBL-CH
- genotyping of 40 lines for anthracnose tolerance
- development of genomic selection and GWAS relative to marker-trait associations by CREA and FiBL-CH

Finally, CREA is developing a population of sweet-set lines by evolutionary selection under autumn sowing in northern Italy. The mild-winter conditions of the last two generations of population evolution in northern Italy make this material potentially suitable for further evolutionary selection steps performed by partners under quite distinct climatic and stress conditions (e.g., high soil lime content in the Netherlands, or severe anthracnose infection in Switzerland, under spring sowing; cold tolerance under autumn sowing, in northern Italy; etc.). This material, which will be available for partners before the end of the project, can be useful both as a genetic resource for future breeding and as scientific material for studying shifts in allele frequencies caused by adaptation to specific, severe stress levels. FiBL-CH and GZPK will identify by end of LIVESEED promising breeding lines and cultivars for anthracnose tolerance.

Visit to CREA's drought tolerance evaluation of novel white lupin lines by representatives of Italian seed companies marketing grain and forage legume varieties (May 2019)

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CREA's evaluation of novel white lupin lines under autumn sowing in northern Italy

Figure 2. Activities on white lupin trials and evaluations

3.2. Brassica

Several skype meetings were done and coordinated by the person in charge (from BNN) since September 2017 (started by K. Arp). It took at least one year to establish an efficient network of partners in order to agree on common goals, approach, methodology and a list of traits of interest among the multi-actors as well as to find volunteers and farmers and cooperatives interested in performing trials in Southern Europe. Thus, in summer 2018 a first list of templates for trials evaluations (Thomas Kimmel from BNN in charge) were agreed on among partners including BNN representing the traders for i) cabbage (white, both round and pointed, and red), ii) cauliflower, iii) broccoli and iv) kohlrabi. These templates included three blocks of traits: at transplanting stage (e.g. germination vigour, germination efficiency, plantlet vigour, etc.), from development to harvesting time (date 50% production harvested, leaf sizes, leaf coverage, leaf colour, heads size, firmness, etc.) and postharvest. Also, a list of potential varieties to evaluate was agreed and the locations for 2018-2019 trials (starting fall 2018).
Moreover, a list of currently available commercial cell fusion-free cultivars was coordinated and elaborated by BNN, Thomas Kimmel in close collaboration with FiBL-CH and organic label Bioland, Naturland, Bio Austria, Bio Suisse, and Demeter. Also, in winter 2018-2019, BNN lead the development of a document relative to the state of the art and currently available cell-fusion free cultivars in EU Southern countries. This list is called a positive list: e.g. a list with cell-fusion free cultivars.
To expand the list of cell fusion-free cultivars, a survey is currently being carried out at various seed companies in 2020 in order to find out to which countries seeds of cell fusion-free varieties have been delivered in the Mediterranean regions. In a second step, the recipients of the seeds are contacted to collect information about their experience with the cultivated varieties on site.
In the winter-spring season 2018/19, trials were conducted in Spain, Italy, Portugal and Brittany. In Spain (Valencia) the trials provided some preliminary information in cauliflower and cabbages (9 varieties in total). Cauliflowers showed an erratic behaviour in comparison to the reference Skywalker F1 (heterogeneous head development and harvesting period, low vigour, high trend to flowering very fast in February, etc.), while some white cabbages showed some potential, particularly the pointed ones. Red cabbages were very slow in development and to reach harvesting period. Additionally, some results from Italy and Portugal provided information of interest in kohlrabi and broccoli. Kohlrabi was evaluated in Italy (Sativa), Portugal (LSSV) and Brittany (ITAB). The results from Portugal were not considered due to bad trial conditions. In Italy (Verona), the Sativa open pollinated populations (OP varieties) (Dario, Sat22) were only slightly later than the reference varieties Lippe F1 and Quickstar F1 and showed a high percentage of harvestable bulbs. The Kultursaat OP varieties KS-JJ-NOR, KS-JJ-TRe37 and KS-CHe-Soko were much later than the reference varieties, so that their percentage of harvestable bulbs resp. their weight were lower. In Brittany, 36 varieties were evaluated (OP varieties,

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F1 hybrid and genetic resources). Most of the commercial varieties (F1 hybrid and OP varieties) were marketable. Sativa varieties (Sat 21, Sat 22) and Bingenheimer Saatgut (BGS) varieties (KS-KOK-JJ-Nor 2014, Fridolin) showed good results compared to variety tests (aspect, percentage oh harvested bulbs, weight). Broccoli was evaluated in Italy (Sativa), Portugal (LSSV) and Brittany (ITAB). In Portugal, F1-varieties showed higher percentage of harvestable heads than OP-varieties. Rasmus was the best OP-variety, showing similar percentage of harvestable heads in comparison to the F1-varieties. The results in Italy were similar. In Brittany, OP varieties, and F1 varieties (some were CMS varieties) were evaluated. Even if the trial suffered from drought, F1 varieties showed higher percentage of harvested heads and individual head weight than OP varieties. Markus F1, and Marathon F1 to a lesser extent, were better than the cell fusion derived CMS hybrid. Moreover, OP varieties have not met the demand of retailers with respect to head aspect, homogeneity, and colour.
In 2019, following the 2nd LIVESEED meeting (Poland) a new list of initiatives and farmers for 2019-2020 trials was agreed and the four different crops were assigned among collaborators and locations. A total of 8 resp. 3 kohlrabi OP- resp. F1-varieties and 4 broccoli OP- resp. F1-varieties were available for testing among partners in cabbage, kohlrabi, cauliflower and broccoli, respectively. Also, an experimental design was agreed for evaluations and the list of traits in templates was improved by adding organoleptic evaluation (when possible).

- Trials in France (ITAB in charge, in collaboration with P.A.I.S.)
- Trials Italy (SATIVA in charge): Kohlrabi
- Trials in Portugal (LSSV Sementes Vivas in charge). Kohlrabi and broccoli
- Trials in Spain (UPV in charge). Kohlrabi, cabbages, cauliflower and broccoli

The main results at the beginning of spring 2020, i.e. winter-spring growing season, were:

In Spain, a total of 6 cabbages (3 white, incl. 2 pointed, and 3 red) and 4 cauliflowers, both cell fusion free OP varieties and breeding lines, plus Skywalker F1 included as cell fusion free commercial control (*Table 1*), were evaluated in two locations: i) Picassent (20 km southern Valencia) and ii) Villamartín-Cádiz (La Verde Coop.). In the latter, other local varieties grown commercially by the Cooperative in the last years were also observed as controls. All materials were transplanted at the end of October 2019 and at least two plots per accession were displayed on each location. Similar results and conclusions were achieved in both experiments/locations. **Cauliflower** OP varieties showed a very poor development in comparison to Skywalker F1, which resulted in small heads. Moreover, within each accession they showed a heterogeneous behaviour in terms of development and the moment of head formation and harvesting. Many plants developed very slowly in comparison to Skywalker F1. Some accessions even flowered very fast with a small (< 10 cm) diameter. Nevertheless, this trend was variable among accessions and some of them were particularly heterogeneous. On the contrary, Skywalker F1 showed uniform plant and head developments and commercial heads. At the end of February-beginning March Skywalker F1 heads were harvested and most were marketable, while only a few heads from OP varieties were harvested and the size was quite small, other plants still had not developed heads and others had flowered before being commercial. Regarding **cabbages**, the evaluated accessions also showed a slow development like cauliflowers, although the uniformity was much higher. The first commercial heads were harvested at the beginning of March in both Valencia and Cadiz (130 days after transplanting). Pointed cabbages were the earliest ones, followed by round cabbages, while red cabbages started harvesting at the end of March (150 days after transplanting). In comparison, local varieties (controls) grown in Villamartin location were harvested since February as usual and therefore only pointed cabbages appear as competitive in terms of earliness, size (small-medium) and taste (sweeter). These results confirm those observed in 2018/19 trials in Valencia with the same collection.

Table 1. List of cell fusion free Brassica OP varieties, breeding lines and F1 hybrids plus cell fusion derived F1 CMS hybrids evaluated in Spain 2019/20

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Crop, accession	Source	Crop, accession	Source
Cabbage		**Kohlrabi**	
212 (Ersteling), pointed	Kultursaat/BSAG	Fridolin	Kultursaat/BSAG
649 (Nagels Frühweiß), round	Kultursaat/BSAG	Noriko	Kultursaat/BSAG
686 (Berns), pointed	Kultursaat/BSAG	Rasko	Kultursaat/BSAG
207 (Amarant), red round	Kultursaat/BSAG	Soko	Kultursaat/BSAG
209 (Granat), red round	Kultursaat/BSAG	SAT79 hetero	Sativa
210 (Rodynda), red round	Kultursaat/BSAG	SAT79 homo	Sativa
Col Hierro	Local source	Korist F1	Bejo
Local 2	Sevilla nursery	Kordial F1	Bejo
Cauliflower		**Broccoli**	
202 (Nuage)	Kultursaat/BSAG	Calinaro	Kultursaat/BSAG
697 (Amabile)	Kultursaat/BSAG	Rasmus	Kultursaat/BSAG
700 (Daniel)	Kultursaat/BSAG	TH Lim 37/59	Kultursaat
KS-KOb-JJ-SG2	Kultursaat	CHE Balimo B	Kultursaat
Skywalker F1	Bejo	Marathon F1	Sakata
		Parthenon F1 CMS derived from cell fusion	Sakata

Regarding kohlrabi, a total of 6 cell-fusion free OP varieties and two commercial cell fusion free F1 from Bejo (Korist F1 and Kordial F1 grown usually by farmers in the location) and 4 broccoli OP varieties and breeding lines and commercial F1 hybrids from Sakata (Marathon F1 and Parthenon F1 CMS derived from cell fusion, *Table 1*) were evaluated in the Campo de Cartagena Shire, Murcia, in fields from SURINVER associated farmers. Two transplanting dates were used: week 42 (14-20 October) and week 48 (25-30 November) for kohlrabi and week 43 and week 49 for broccoli, and two plots per variety were displayed per transplanting date. **Kohlrabi** OP varieties showed in general a good behavior in comparison to commercial F1 controls, although differences among varieties were found (*Figure 3*). Thus, some varieties showed development and head formation earliness and uniformity comparable to those from Korist F1 and Kordial F1: Fridolin, Soko and particularly SAT79 hetero can be considered as commercial alternatives, while others like SAT79 homo and Rasko were very heterogeneous and or very poor and slow in development. Apart from TH-Lim37/59 with lower vigour, **broccoli** varieties showed good plant uniformity, vigour and development similar to those of Marathon F1 and the cell fusion derived Parthenon F1. However, the head development and formation were less uniform (*Figure 3*) than the commercial controls and therefore less competitive from the perspective of SURINVER producers.

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Kohlrabi pre-evaluations in Spain (end Nov. 19), Korist F1 (center) and Noriko (right)

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Broccoli trials in Campo de Cartagena (Spain) at the pre-evaluations (Upper row, end Nov. 19), and from the second row to the bottom: comparison of developing heads from Parthenon F1, Rasmus, Balimo and Calinaro, 1 month prior to harvesting (mid Jan. 20)

Figure 3. Organic cell fusion free Brassica vegetables trials in Spain.

In Portugal, Sementes Vivas performed the evaluation of kohlrabi and broccoli in the winter-spring season 2019-20. Regarding kohlrabi trials, eight OP-varieties and breeding lines from Kultursaat and Sativa were evaluated together with three cell fusion free F1 varieties from Bejo (Korist F1), Sakata (Quickstar F1) and Rijk Zwaan (Lippe F1) in Herdade do Couto da Várzea (*Table 2*). Plants from each variety were distributed in several plots (2-5), with three different sowing dates (6 and 24 of September and 1 of October) and two different transplant dates (22 and 29 of October) for both crops. The best results were found in the first transplanting date/second sowing date. The suboptimal results of the first transplanting date/first sowing date are related to negative conditions at nursery leading bad quality seedlings. The second transplanting date/third sowing date are clearly too late in Portugese conditions to give good results.
In general terms, **kohlrabi** crop performed quite well, with some open pollinated varieties having yields very similar to the F1 used, such as Fridolin, SAT79 homo, SAT79 hetero and Enrico, with real emphasis on the latter. In contrast, Soko and Rasko varieties did not show a good performance in our conditions, with a very low yield, and accordingly a very low number of marketable bulbs.
Regarding the **broccoli** crop, in contrast to what happened in kohlrabi, there were already significant differences between the vegetative development of F1 and open pollinated varieties (Rasmus and Calinaro), with less vigour in the latter ones. The F1 showed uniformity in the development of the heads, leading to a harvest with greater yields, larger and heavier heads, in addition to being more compact, resisting bolting well even for later harvest. The two OP varieties, despite having developed reasonably in vegetative terms, especially showed reduced performance during head formation and uniformity of maturation. On top of this there were a very low number of marketable heads, a low weight per head, very few compact heads and a very fast bolting.

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Table 2. List of cell fusion free Brassica OP varieties and F1 hybrids plus cell fusion derived F1 CMS hybrid evaluated in Spain 2019/20in Portugal

Crop, accession	Source	Crop, accession	Source
Kohlrabi		**Broccoli**	
Dario	Sativa	Calinaro	Kultursaat/BSAG
Enrico	Sativa	Rasmus	Kultursaat/BSAG
Fridolin	Kultursaat/BSAG	Belstar F1	Bejo
Noriko	Kultursaat/BSAG	Marathon F1	Sakata
Rasko	Kultursaat/BSAG	Parthenon F1 CMS Cell fusion derived	Sakata
Soko	Kultursaat/BSAG		
SAT79 hetero	Sativa		
SAT79 homo	Sativa		
Korist F1	Bejo		
Lippe F1	Rijk Zwaan		
Quickstar F1	Sakata		

In Italy, a **kohlrabi** trial was conducted on a bio-dynamic farm in Verona. Three Sativa lines (Enrico, Sat79 hetero, Sat79 homo) were compared to the cell fusion free variety Lippe F1 (Rijk Zwaan). The trial was harvested in once, as it is commonly done in professional production. Lippe F1 showed the highest percentage of harvestable bulbs (96%), followed closely by Enrico (87%) and further both Sat79 hetero and homo (83% and 82%, respectively). The trial confirmed earlier experience from SATIVA that Enrico has quite a good performance, with ca. 10% less marketable yield than standard hybrid varieties.

In France, kohlrabi, broccoli and cauliflower trials were conducted at the experimental farm of P.A.I.S. in Brittany (*Table 3*). 2 sets of **kohlrabi** trials were conducted under poly-tunnels during the winter-spring season. First plantation period was in October (the 16th), and the second was at the end of January (the 31st). For the first set of trial, sowing and planting were too early for Brittanian winter, followed by a warm spring induced early flowering for all the varieties but one, so that no harvest could be done. For the second trial planting in January, 36 accessions from genebank and several varieties were evaluated. Some of the genetic resources BRA 1889 and BRA 2151 4 (violet type) and BRA 2086 (green type) gave similar results to other OP varieties (Fridolin, Sat 21).

For broccoli and cauliflower, drought after planting reduced yield for every variety tested. For cauliflower, few plants produced marketable heads, but some plants of Nuage and KS KOB JJ SG gave promising results in comparison with Skywalker F1. Drought particularly induced early flowering for some broccoli varieties. Head quality (flower bud size, head firmness, homogeneity), and size were not sufficient enough for Brittany producers and retailers for OP varieties. Some plants for Calinaro (and to a lesser extent SAT 30 and SAT 31 *Figure 4*) produced heads with a quality similar to F1 hybrid Marathon, but lower than Steel F1 (reference for cell fusion free hybrid in Brittany) and Marcus F1 (cell fusion derived CMS hybrid).

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Table 3. List of cell fusion free Brassica OP varieties, breeding lines and F1 hybrids plus cell fusion derived F1 CMS hybrid evaluated in France (P.A.I.S., Brittany)

Crop, accession	Source	Crop, accession	Source
Cauliflower		**Broccoli**	
Nuage	Bingenheimer Saatgut	KSV LIM 20/68	Kultursaat
KS KOB JJ SG	Kultursaat	KSV LIM 19/28	Kultursaat
KS KOB JJ REV	Kultursaat	KSV LIM 37/59	Kultursaat
KS KOB MG AMB	Kultursaat	SAT 30	SATIVA
Skywalker F1	Bejo	SAT 31	SATIVA
		SAT 32	SATIVA
Kohlrabi		Marcus F1 CMS cell fusion derived	Voltz
36 Genetic resources	Gatersleben genebank	Belstar F1	Bejo
Dario	Sativa	Covina F1	Bejo
Enrico	Sativa	Marathon F1	Sakata
Fridolin	Kultursaat/BSAG	Steel F1	Seminis
Noriko	Kultursaat/BSAG	Calinaro	Kultursaat
Rasko	Kultursaat/BSAG	Haitabu	saat:gut
Soko	Kultursaat/BSAG		
SAT79 hetero	Sativa		
SAT79 homo	Sativa		
Korist F1	Bejo		
Lippe F1	Rijk Zwaan		
Quickstar F1	Sakata		

Seeds of organic bred broccoli varieties have also been provided to the sister project BRESOV working on broccoli, tomato and bean breeding for the organic sector. Thus, seed source of promising cultivars were also provided to BRESOV for the establishment of a network of on farm trials across different European countries. Complementary to the participatory approach of LIVESEED, BRESOV is heavily involved in phenotyping and genotyping to map genes for drought and several resistance traits and marker assisted selection. However, they mainly work on inbred lines for hybrid development which might hamper the exchange of material with organic breeders.

Kohlrabi harvested bulbs (from left to right): KS-KOK-77-NOR, SAT 21 and Fridolin

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Broccoli trial at P.A.I.S. experimental farm (Brittany, France) (left), and heads from SAT 31 (center) and plant and heads from SAT 30 (right)

Cauliflowers in plants in P.A.I.S. experimental farm trials (Brittany, France) from KS KOB JJ SG (left), Nuage (center) and Skywalker F1 (right)

Figure 4. Organic cell fusion free Brassica vegetables trials in France (Brittany)

Results of the LIVESEED trials were presented at several national workshops in exchange with the whole value chain. This is important to communicate that OP varieties will not reach the same level of homogeneity than an F1 hybrids. Thus, traders also need to compromise their expectation on homogeneous heads if they want to have cell fusion free varieties.

3.3. Apple

Poma Culta (linked third party of FiBL-CH) has started organic apple breeding and selection under biodynamic participatory approach 25 years ago producing about 5000 seedings derived from new crosses each year. In the scope of LIVESEED Poma Culta has 2017 established and coordinated the set up of a network to test six of the most promising apple candidates (TEMA) across Central Europe (CH, IT, DE, NL) in order to gain experience if the candidates are accepted by organic growers. Trees are integrated into a barcode system for annual assessmets. In 2019 first fruits could be harvested from these pilot trials. While some candidates were quite promising like No 3800 (*Figure 5*), two were discarded due to severe scab infestation or low yield. They will be replaced by two new candidates in 2020. FiBL-CH is involved in organic apple cultivars assessment and development of breeding tools in close collaboration with Poma Culta and Agroscope (co-financed via Swiss project) with focus on quantitative resistance of scab and new emerging diseases like fire blight and Marsonnia as well as fruit quality testing and storability. This involves the establishement of novel phenotypic screening

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tools and also analysis of published markers (e.g. developed in FRUITBREEDOMICS and RosBREED) for resistance and fruit quality traits. FiBL-CH and Poma Culta also conduced sensory testing with semi-trained panel and consumers at different local events in Switzerland. The organic apple breeding project was also presented with degustation of candidate apples at the biggest organic faire biofach Messe Nürnberg 2018 and 2019.

Figure 5 Apple candidate cultivar 3800 at organic farm in the Netherlands in 31.07.2019.

A broad network of organic apple breeding actors has been already created connecting various research and breeding projects from all over Europe (Switzerland, Germany, France, Belgium, Spain, Greece, Italy, Denmark, Austria, Czech Republic, Romania, Portugal, Finland, Serbia). The network is consisted of breeders, apple experts, nurseries and growers from all over Europe (LIVESEED apple network). The different apple breeding and testing actors have been mapped by following activities:

- Ten LIVESEED skype minutes and annual meetings with LIVESEED apple task partners (AEGILOPS, FIBL-CH, PomaCulta, GRAB, ITAB) in order to clarify targets, integrate literature or updated information and plan networking activities
- Four LIVESEED Apple task workshops conducted at 18th and 19th Eco fruit Conferences, Biofach 2019, and 28th AGROTICA
- Online apple task survey launched in July 2018: 17 responders (breeders, nurseries, farmers, experts etc) from different European countries
- Participation to breeding and relevant scientific conferences and meetings: poster and oral presentations to 18th Eco fruit Conference 2018 (Germany), poster presentation to Fruitculture symposium in Portugal, (November 2018), poster presentations to 2nd European Agroecology Forum, (Crete, Greece – 26-28/9, 2019), EGON Research Project fruit breeding meeting (Germany -November 14, 2019)
- Organization of informative and demonstrational events/meetings: Poma Culta field day (July 2018), AEGILOPS' 15th Anniversary field day (July,2019), AEGILOPS information events in Lesvos Island and Volos (Greece, autumn 2018) in cooperation with Greek Pomology institute during fruit conservation expeditions
- Personal contacts. Other key stakeholders were also connected: apple experts, nursery holders, farmers, market actors, policy makers and breeders of peach, apricot in Switzerland

This revealed quite a number of ongoing apple breeding, testing and cooperation initiatives in national, regional and European levels published on the LIVESEED homepage. This was the first step to foster future collaboration.
Dynamic state of art has been developed including breeders, contact information activities, breeding targets, candidate cultivars and rootstocks, existing and ongoing breeding projects and stakeholders

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involved, market priorities and farmers´ needs. The contribution of old local varieties to broaden the genetic basis in breeding has been stressed by initiatives in many European countries involved in phenotypic screening and testing evaluation under low input or organic conditions of many traditional apple accessions (France, Switzerland, Italy, Greece, Lithuania, Sweden). In **Switzerland**, Poma Culta, supported by FiBL-CH is involved in organic apple breeding and cultivars testing in collaboration with Agroscope. Moreover, FiBL-CH is involved in the evaluation of new apple cultivars under organic conditions and developed a marketing concept based on categories of taste (sweet – semi-sweet-acidic) and ripening time. Before new cultivars will finally be recommend for organic production, a group of organic growers has tested them under field conditions and the results have been discussed with stakeholders of the whole organic apple market chain (Friedli and Messmer 2019). Organic Apple breeding activities in **France** involve cooperation between LIVESEED partners INRA, ITAB, GRAB and a broad network of farmers who are testing candidate varieties. The organic breeding project called Novafruits is led by CRA Gembloux in **Belgium**. In **Germany**, EGON project is a joint national research project between Oldenburg University, Apfel:gut e.V. and Oeko Obstbau Norddeutschland e.V. to investigate the development of organically bred fruit varieties in commons – based initiatives. Apfel:gut developed organic fruit varieties with a participative approach. After an evaluation, robust and high yielding old cultivars are mainly crossed with less susceptible new varieties. Additionally, old cultivars are crossed with other old cultivars and open-pollinated seeds are sown from time to time. Through the use of varieties as donors, which are not closely related to modern varieties, the project expected to increase genetic diversity of organic apple cultivation. In a joint effort, the former EU project ‘Fruitbreedomics’ and the US project ‘RosBREED’ contributed to increase efficiency in marker assisted breeding, a highly useful approach towards successfully pyramiding of resistances in scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*). Research institutes in middle Europe also started trials on the comparison of rootstocks, which should be less susceptible to fire blight. In the Asturian (NW Spain) agrarian sector, where apple production is principally geared towards cider making, research on sustainable strategies for managing cider-apple, cultivars selected by SERIDA since 1986 are highly recommended because of their disease tolerance, productivity and juice quality.

The need for long term coordination among actors and projects and long-lasting networks to save, spread exchanged knowledge, data as well as genetic material was expressed from various breeders. While such network exist for conventional fruit production like EUFrin (European Fruit Research Institutes Network) connecting institutes in Europe on fruit research, which involves working subgroups in interaction with EU Commission and common agreements between breeders on variety tests, similar network is missing for the organic sector. Therefore, LIVESEED initiated the discussion of founding of a “European Participatory Cooperative Network of Organic Apple/ Fruit Breeding and common Database”. This can become the basis for trustful collaboration for the benefit of all actors. Especially the exchange of vegetative planning material of new candidates will only be possible through personal relationships and trust.

Ongoing activities:

- Evaluation of agronomic performance, taste, storability, and horizontal resistance of 6 candidate clones in organic farmers conditions in CH and Central EU countries (Poma Culta, FiBL-CH)
- Investigation of opportunities for organizing an Organic Fruit Breeding Meeting for South East European and Balkan countries in Greece on autumn 2020, in cooperation with Greek Pomology Institute (HAO Demeter)
- A meeting on common database needs was suggested to be organized in summer or fall 2020. Niklaus Bolliger (breeder and CEO of Poma Culta, CH) offered to host a small group in order to further discuss organization details, constrains and needs
- A relevant electronic booklet and/or collective video explaining difficulties and assets of breeding for organic farming
- Preparation of the founding of European Participatory Cooperative Network of Organic Apple/ Fruit Breeding and common Database

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3.4. Winter wheat

The main work in this Task 3.4.4 so far has been the field assessment of the field trial assessing the resistance of 2700 genotype-virulence combinations for common bunt. The field trials of 270 genotypes derived from introgression of Bt1, Bt2, Bt5, Bt7, Bt13, BtZ and Quebon resistance genes with 10 different virulent strains of common bunt have been conducted in season 2017/18 and 2018/19 by AGROL in Denmark. Results showed that it is indeed possible to group the breeding lines and varieties according to their resistance consistent with the hypothesis based on the resistance of the parental lines used for crosses. The results from the first experimental year are published in Borgen et al. (2018). The result from the second experimental year are being processed and have been disseminated to Task partners for comments and planning of further crosses.

The 450 breeding lines, including 30 lines from the German breeders Dottenfelderhof and Cultivari, have been sent to UNI KASSEL in 2019. Based on the results from the field trials of 2018/19, 288 lines are selected for genotyping with 25K chip. DNA from these lines were extracted and sent to the TraitGenetics GmbH in the spring 2020 to obtain SNP data based on genotyping by sequencing (GBS). The resistance data and genotyping data will be used in a genome-wide association study (GWAS) in order to identify links between marker loci and trait expression (UNI KASSEL). GWAS procedure for the wheat has been already established at the UNI KASSEL within the INSUSFAR Project and will be adjusted and used for the obtained genotyping. Several statistical methods for association are being used like general linear model (GLM), mixed linear model (MLM), multi-locus mixed model (MLMM), fixed and random models circulating probability unification (FarmCPU) using the R script GAPIT version 3, as well as a network-based model using the R package netgwas for detecting epistatic selection. In order to avoid false positives, GWAS methods use information about population structure that is calculated using principal component analysis based on filtered SNPs, as well as principal coordination analysis, M-spline multiple dimensional scaling using MM algorithm with Torgerson initial configuration, uniform manifold approximation and projection using spectral embedding, and also PCoA-UMAP based on clumped SNP. Filtering and clumping are performed for the genetic maps as described by Allen et al. (2017; wheat breeders' array), Gardner et al. (2016), and Wang et al. (2014). All calculation except the SNP filtering are performed in R statistic environment. The SNP filtering were performed in Microsoft Excel using a macro written by Gunter Backes UNI KASSEL.

Data obtained by GWAS should result in a mapping of new and effective resistance genes and developing of genetic markers for selection of these genes. The most promising lines derived from the first screening will be tested in the season 2020/21 at the sites of the two German wheat breeders in small plots for agronomic traits and baking quality at very different pedo-climatic conditions (Dottenfelderhof and Cultivari). There is also frequent exchange with the researches of BOKU of the EU sister project ECOBREED on application of markers for bunt tolerance in order to use synergies between the two projects aiming to improve bunt resistance by pyramidizing different resistance genes. As LIVESEED is focusing on Bt1, Bt2, Bt5, Bt7, Bt13, BtZ and Quebon resistance sources and ECOBREED on Bt9, Bt10, Bt12 (Muellner et al. 2020) the projects complement each other nicely. LIVESEED breeding partners also shared their most promising wheat lines with ECOBREED partners.

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In 2019 AGROL, Dottenfelderhof and Cultivari participated at the International Cereal Diversity Festival in Denmark 2019, including a workshop specific on common bunt, where they presented their work in a shared session their cereal and bunt breeding programs. In addition, they have visited the trial site and the field experiments organized in cooperation with Task 2.2.2. AGROL have organized 3 open field days in LIVESEED field trial in 2019.

Planned activities:

- **XXI International Workshop on Bunt and Smut disease** will be conducted in 26-27. Nov 2020 in collaboration with the EU Project ECOBREED at the University of Natural Resources & Life Science, Vienna (BOKU), Campus Tulln, 3430 Tulln, Austria attached to the Annual meetings of Austrian seed associations in Gumpenstein

3.5. Tomato

In Task 3.4.5 on participatory tomate breeding towards local adaptation have been conducted in parallel in Spain by UPV and in Italy on composite cross population (CCP) developed during former Solibam project by RSR and Arcoiris and on a MAGIC population by CREA and respective farmers and value chain partners in the different regions. Below are the major outline of the three PPB tomato breeding networks for local adaptation that cover three different plant populations and approaches:

- **PPB progam 1, landraces in 3 regions in Spain**: Broad collections of 250 Spanish landraces assessed in two main areas: Sagunto-Valencia (Mediterranean) and Villamartin/Cádiz (Andalusia). 120-150 accessions per year distributed in two locations, 20 plants/accession and location). UPV in charge
- **PPB program 2, CCP in 5 regions in Italy**: CCPs derived populations from “cuore di bue” tomato assessed in five locations of Italy (400 plants/location on first year, 800 plants/location on second year and comparative trials of all the selection in all locations – 560 plants/location - during third year). RSR and linked third party Arcoiris in charge
- **PPB program 3, MAGIC population 3 regions in Italy**: experimental MAGIC (multiparent advanced generation intercross) population (370 G4 plants) and other 55 lines (5 Spanish + 20 Italian landraces + 30 already selected MAGIC plants) submitted to PPB in 3 organic farms (North, Central, South). CREA Monsampolo in charge

To date, two PPB yearly evaluations have been done in the three PPB programs under open field conditions in organic farms: 2018 and 2019 spring-summer seasons, in both countries.
Participatory evaluation was conducted by different actors involving (*Figure 6*):

- Farmers/breeders and technicians from cooperatives, organic nurseries providing transplanting on general appearance
- Scientists and technicians on yield, growth habit, incidence of pest and diseases
- Lab technicians on sugar, acids, total phenolics
- Preselection of most promising lines conducted by the involved actors
- Consumers and traders on optical appearance, fruit quality and organoleptic characteristics of preselected lines and their acceptance by consumers.

Within PPB program 1, about 250 landraces have been evaluated in the two years 2018 (130 accessions) and 2019 (120 accessions) distributed in both main locations Sagunto-Valencia (organic fields of La Unió Association) and Villamartín/Cádiz-Andalusia (La Verde Coop.):

- 2018: 130 accessions, 100 Villamartin and 80 Sagunto, 25 in common both locations
- 2019: 120 accessions, 90 Villamartin and 80 Sagunto, 20 in common both locations

On-the-field participatory evaluations have included a range of agronomic traits like yield, fruit shape and size, plant vigor and development, leaf coverage, management, incidence of pests and diseases

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(e.g. Tuta absoluta, TSWV, ToMV, fungal diseases like verticillium, etc.) and physiopaties (e.g. sunscald, blossom-end-rot) for which evaluations templates have been developed. Also taste-related traits like sugars and acids content were evaluated.

According to these evaluations, several accessions were preselected for taste evaluations with consumers and retailers in open days activities. During these activities, some diffusion of the project and the aim of the tomato subtask was also done to spread to the general public the interest of the initiative. Also tomato organoleptic evaluation with the audience was done in order to i) include consumers´ preferences as key selection factor and ii) show the diversity of tomato landraces to the general public.

After the two years of evaluations, about 40 accessions were preselected for each location (800-1000 plants). These landraces are presently re-evaluated in PPB in year 3 (2020), following the same criteria mentioned above, for a final selection of about 10-20 accessions per location. Complementary analyses of the volatile fraction and antioxidants are planned for the selected tomato landraces. Over all analysis of the three years assessment and comparison between farmers' and consumers' preference with morphologic, resistance and yield related traits and fruit composition.

In this PPB program 1, two evaluation templates have been developed for tomato: i) for participatory agronomic evaluation and ii) for organoleptic evaluation with consumers. A video shows the consumer involvement in tasting. Also, dissemination activities with general public/consumers have been done in both locations in the open days and local markets in years 2018 and 2019. Finally, some contributions to congresses and workshops have been also done (Feria Biodiversidad Valencia 2017, SEAE 2018 General Congress, Workshop on Opportunities for Organic Seeds in Almeria 2019) and video recorded activities of open days and tomato seed extraction in 2019 Valencia trials available in YouTube.


Tomato PPB-social activities with general public and consumers´ evaluations. Posters for announcements of activities in summers 2018 and 2019 in Valencia (left and right) and consumers evaluations in Mercado de los Toruños (Puerto de Santa María, Cádiz, August 2018, La Verde Coop selling point)


Tomato PPB activities in Italy. Locations of farms (left) and groups or evaluators coordinated by RSR and Arcoiris

Figure 6. Tomato participatory trials and social activities in Italy and Spain.

Within PPB program 2, two cycles of participatory selections were carried out Composite Cross Populations "Cuore di Bue" tomato (i.e. ox heart) are being evaluated and selected by farmers under organic condition in five different locations in 2018 and 2019. The selection was carried out on 400 plants per location, grown under organic conditions and according to the management prevalent on each farm.

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- In 2018, on each location, the 20 best plants were selected after participatory evaluation by local farmers (value of cultivation based on agronomic quality), whilst one fruit per plant was collected to maintain the overall genetic diversity of the population.
- In 2019 on each farm two separate 400 plant plots were established: one deriving from the complete population (natural selection); the other from a balanced population (farmer selection) created by mixing equal number of plants deriving from the 20 best plants selected the previous year. The participatory evaluation was conducted on the "farmer selection" population, and seed collected from the best 20 plants, as well as from all of the 400 plant of the "natural selection" population. Because of complete crop failure in one of the farms, the number of location was reduced from five to four. This meant that by the end of 2019 we had generated:
    - 4 random population (natural selection)
    - 4 farmer selection population (farmer selection)
- In 2020 comparative trials were set up in all four locations, in order to assess whether specific adaptation with measurable effect had occurred during the two previous growing cycles and round of selections. This was done by setting up a randomised block design with two replications including:
    - 4 natural selection SOLIBAM Cuore di Bue populations
    - 4 farmer selection SOLIBAM Cuore di Bue populations
    - 4 local varieties chosen by farmers as their local check (one per location)
    - 1 open pollinated modern variety chosen by farmers as control
    - 1 Cuor di Bue type F1 hybrid chosen by RSR in cooperation with the breeder (ISI sementi)

At the time of publication of this report the 2020 trials are been set up and experimental field planted. Agronomic traits will be collected during the growing season (vigour, disease resistance, uniformity, yield), and a participatory evaluation will be carried out by farmers at harvest time.

Within PPB program 3, during the two-project year 2018 and 2019 were evaluated a different number of genotypes derived from the originally MAGIC population, (based on 8 divergent parents and encompassing a range of fruit qualities, salt and cold tolerance, resistances to fungal and virus diseases) under partially replicated experimental scheme in three geographic distinct regions.

- 2018: 400 MAGIC plants (G4, 4th generation) were cultivated together with the 30 plants selected the previous year, both at the CREA on the MOVE LTE device according to the cultivation system of conservative agriculture and at 3 organic farms in Northern, Central and Southern Italy
- 2019: 20 plants derived from each plant selected in the 2018 on the basis of participatory breeding and marker assisted selection (MAS) for resistance to pathogens, shape and colour of the fruits, were grown at the same farms as in previous years.

During the 2018 summer season, at CREA the material was visually phenotyped only by the researchers. The participatory plant breeding was carried out at the 3 organic farms. In all locations, the trials were conducted by applying local cultivation techniques to produce tomato following the agronomic practices indicated by the European organic legislation. The participatory selection was carried out by a team composed of farmers, technicians and researchers, who individually assigned the following score to the plants: 1 = not at all satisfactory, 2 = unsatisfactory, 3 = satisfactory, 4 = very satisfactory. The parameters considered in the evaluation were: growth habit, plant vigour, health, yield, fruit shape, fruit size, fruit colour, homogeneity of ripeness, fruit firmness, flavour. Based on the score obtained, the fruits of the best plants were collected, and the seeds were extracted to be planted in the 2019 summer season.
During the 2019 summer season, 20 plants per each previously selected plant (year 2018), for a total of 400 plants, were cultivated at the 3 organic farms. PPB was carried out on all the cultivated material, in order to assess the homogeneity of the "family" (typology correspondence to the original selected plant) and to select new single interesting plants.
The considered parameters to which a score of min = 1, max = 5 were attributed, were:

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- on the plot basis: homogeneity, health, plant vigour, abundance and uniformity of fruiting, overall agronomic judgment on the parcel
- on the selected plant within the plot: growth habit (determined, semi-determined, indeterminate), health, plant vigour, abundance and uniformity of fruiting, fruit shape, fruit size, fruit colour, fruit box, Brix °, fruit weight (g)

Unfortunately, due to environmental adverse condition the trial carried on at Padua (North) failed. After the two years of evaluations, 23 families and 43 individual (G6) plants were selected. In particular, the number of plants selected was different among the locations:

- Central (Fermo): 9 families and 10 individual plants
- South (Matera): 10 families and 16 individual plants
- North (CREA): 4 families and 17 individual plants

All the selected materials together with the 8 parental lines and 500 (G10=10th generation) plants obtained by ISI Sementi from the original MAGIC population by single seed decent (SSD) during the project, will be genotyped by SPET (Single Primer Enrichment Technology) to identify new molecular markers associated to important agronomic traits and study the shift in allelic frequencies in the populations cultivated at the 3 different soil and climatic locations to understand the effect of participatory selection and environment on the original MAGIC population.

The activities developed during these two years were spread to the general public in open days, workshops and congress also related to the BRESOV H2020 project (XIX EUCARPIA meeting of the Tomato Working Group, Napoli 2 - 4 May 2018; LXII SIGA Annual Congress, Verona 25-28 September 2018). Finally, a paper was published by Campanelli et al. 2019. In addition there quite close exchange between the LIVESEED and the sister project BRESOV as UPV is involved in both consortia and responsible for the tomato breeding activities. This allows to share experience on different approaches and co-create knowledge. While LIVESEED is focussed on PPB tomato breeding for local adaptation and consumer preference, BRESOV is focusing on introgression of resistance genes and nutrient contents.

4. Preliminary achievements and challenges of each subtask/species

4.1. White lupin

At the beginning of the project, the partners agreed on the key importance of broadening the genetic base of this crop. The project made a remarkable effort in this direction, setting up a crossing program between elite sweet-seed and elite landrace germplasm, producing a large number of progeny lines, distributing over 140 progeny lines for evaluation of adaptation to several environments and/or key stresses and grain quality traits and, at the end, producing out of the same crosses an evolutionary population of inbred lines for future breeding work by partners. Only a fraction of the newly developed germplasm has been evaluated for some traits, while the remaining material awaits completely the exploration of its value. All these materials await exploitation by through selection for specific target environments and utilizations. Special efforts were taken to screen large number of landraces for anthracnose resistance by phenotypic selection in order to identify sources for horizontal resistance and markers linked to this trait. The already published markers from Australia were not transferable to Central European conditions. Since white lupin shows a considerable proportion of outcrossing caused by insect pollination (up to 20%), breeding is more cumbersome compared to other strictly inbred species like wheat. Moreover, broadening the genetic base by introgressing landraces or genebank accessions is always associated with reintroducing dominant wild-type genes causing high alkaloid contents. Therefore, developing the population of lines took time and required to assemble partly inbred lines rather than strongly segregating material, because of the need for prior screening

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of partly inbred lines, such as the generations F3 or F4, for low content of alkaloids. In addition, the exposure of bitter-seed wild-type in a breeding program, which have a greater fitness for tolerance to biotic stresses, has the potential to accumulate during selection process. This bears the risk that these genes accumulate in a open pollinated population and thus in the medium term the risk to exceed the allowed alkaloid limit for human food.

These circumstances suggest using white lupin populations mainly as genetic bases for the breeding of pure lines (or mixtures of pure lines) as otherwise the bitterness will increase due to selection advantage. Besides being extremely valuable as a genetic base, the developed population can be a valuable tool for the future study of shifts in morpho-physiological characteristics and allele frequencies that are associated with the adaptation to extreme environmental stresses (e.g., soil lime; severe drought; anthracnose).

At the moment of writing this report, it is still largely unknown the range of variation and the agronomic value of the novel lines, because most of the evaluation experiments need completion and most of the available experimental data were not analysed yet. Preliminary analyses confirmed the occurrence of sizeable genetic variation among the tested sweet-seed lines for yielding ability under drought-prone and moisture-favourable conditions, but very limited for anthracnose tolerance. Molecular markers data have not been entirely obtained from GBS genotyping, while the very recent publication of the white lupin genome in France will make the future SNP marker calling from sequencing data much more reliable. The results from the joint analysis of phenotyping and genotyping data and the performance of GWAS and genomic selection analysis, which will be available by the end of the project, will allow to assess whether molecular breeding could be more cost-efficient than ordinary phenotypic selection for the improvement of complex traits such as drought tolerance and adaptation to moderately calcareous soils, or a crucial trait for organic systems such as tolerance to anthracnose. Results are awaited by the end of the project also on the ability of NIRS-based and genomic tools to predict grain quality traits.

LIVESEED contributed significantly to reduce gaps and bottlenecks that hinder the progress of white lupin improvement work in Europe. Broadening the crop genetic base represented the necessary premise for future improvement. As anthracnose symptoms do not occur in all countries (like Italy, Netherlands) as severity also depends on environmental conditions during lupin cultivation, the development of a reliable screening tool under controlled conditions with isolated and genetically defined strains is an important innovation to better understand plant – pathogen interaction and to boost resistance breeding. Another important merit of LIVESEED was to strengthen the collaboration and the possible complementation of research groups aimed to improve genetically this crop. Such groups are very few worldwide and used to work largely disconnected to each other. The project built up trusts between groups and stimulated future joint work, not only among the three partner breeding institutions (CREA, FiBL-CH, LBI and UBIOS) but also with other public or no-profit institutions working in Europe (James Hutton Institute, UK; Institute of Industrial and Forage Crops, Greece) or in South America (Instituto Nacional de Investigación Agropecuaria, Chile) to which project material was delivered for evaluation and joint research work was set up or which provided genetic material for anthracnose screening (several national genebanks and breeders Eric von Baer (Chile), LfL, DSV (Germany).

While awaiting the end of the project to quantify more precisely the agronomic value of the newly-generated plant material and the breeding tools developed by LIVESEED, we may anticipate that most of the plant material and new tools await future exploitation, ideally by means of coordinated efforts by breeding institutions and other stakeholders. For example, promising tested new sweet-seed lines could undergo further evaluation, especially under organic farming conditions, in view of their introduction into cultivation. Cost-efficient genomic selection and MAS tools could be used to identify genomically promising sweet-seed lines among material that was genotyped but not evaluated, or future breeding lines. The evolutionary population of breeding lines represents not only a valuable broadly-based genetic resource but also a scientific tool, as its future exploitation by various interested partners may contribute to disclose genomic areas associated with plant adaptation to

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specific abiotic and biotic stresses. Future bulk-segregant analysis could be used to validate the findings from the phenotyping and genotyping analysis of many individual lines and perhaps provide a less resource-demanding tool for achieving similar results. It is also expected that LIVESEED will result in interesting candidate cultivars with increased level of resistance. Based on first years data of 2 newly released white lupin cultivars we are hopeful to give already some recommendation of suitable cultivars for organic farming under anthracnose pressure.
LIVESEED was important to reduce gaps and bottlenecks for white lupin by various means. It brought competences together, built trust, and started joint research work among partner research groups belonging to the project and also a few other research institutions of Europe or South America that agreed to add to the project by own work and resources. Some joint work is already scheduled to continue, for example the evaluation in specific cropping environments of the evolutionary population of sweet lines. Also, LIVESEED funded activities for the indispensable broadening of the white lupin genetic base, and allowed for the joint testing of material, which is expected to result in the availability of new cultivars in various countries (which is another well-recognized priority for the crop). Finally, the project offered the opportunity to widen the limited knowledge on genetics and genomics issues, especially with respect to tolerance to key abiotic and biotic stresses, and exploit this information to develop and preliminarily test new molecular marker-based selection tools (whose value will be assessed by the end of the project).
While some joint activities are scheduled to continue in the near future, possible projects may allow to fully exploit the newly-generated genetic resources (including the many non-tested sweet-seed inbred lines and the new lines obtainable from the evolutionary population after evolution in several specific environments), and to validate, refine and thoroughly exploit promising genome-enabled selection tools. Additional research work will be needed also on specific characteristics that are required for white lupin adaptation to organic systems, such as competition against weeds and suitability to intercropping.

4.2. Brassica

The first objective of the Brassica subtask 3.4.3 was to establish a network among the involved partners and stakeholders and exchange experience between Central Europe and Southern Europe. A lot of meetings to discuss approaches, priority setting, revision of cell fusion free varieties and breeding lines available, potential testing locations and involved stakeholders that offered such trials, etc. were necessary to agree on a common strategy and set up trials in Southern European countries. It was important to reach agreement among the numerous partners on a common goal and approach which also refects to different socio-economic context in the different countries (CH, DE, FR, IT, ES, PT). Therefore the first trials started in winter 2018/19 as the right season for Brassica crops in Southern countries is the winter-spring season. This also allowed that all partners used a common set of approaches, descriptors, and many plant materials in common, mostly provided by the organic breeding initiatives Kultursaat/BSAG and SATIVA, together with well known commercial F1 controls. Thus, results can be easily compared and wide or specific adaptations to organic cultivation in different Southern locations can be identified clearly and also compared to those agroecological conditions where these varieties and breeding lines were originally bred.
At the end of the project, with a new evaluation in 2020/21 winter-spring, we will have more robust results, comparisons among countries and conclusions which cell fusion free Brassica species and cultivars were adjusted for various geographic regions and markets. It will also clearly show where further breeding is needed for local adaptation to winter planting of cell fusion free Brassica vegetables. Here are exchange with the PPB approaches of Task 3.4.5 offer a great opportunity to boost up breeding for local adaptation.
At the time of this report, several conclusions can be deduced from the own trials and results of evaluations. Thus, from evaluations in **Spain** we realized that kohlrabi and to a lesser extent cabbages (white and pointed) had an opportunity and caught the interest of farmers and also consumers. Some

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kohlrabi accessions showed a behaviour similar to that from commercial controls Korist F1 and Kordial F1, and some of them with a particular sweet and attracting taste and flavour. In the case of cabbages, despite the fact that pointed cabbages also showed an appealing taste (sweeter and less sulfurous than common cabbages), we realized that all the evaluated materials develop very slowly under the Spanish winter (and produce consequently small heads) in comparison to other commercially available sources, including Spanish local varieties. As a result, new initiatives to find cell fusion free cabbages adapted to organic cultivation should rely in exploring local germplasm and ecotypes for direct use or to develop pre-breeding materials. Finally, broccoli and cauliflowers were found more difficult crops to find cell fusion free materials suitable for organic production under the Spanish/Portuguese/Italian winter season. Moreover, their final use as immature inflorescence and their inherent flowering risk made them a real challenge for breeders. In particular, the cauliflowers tested showed a very erratic behaviour, slow development and small heads in comparison to the commercial reference Skywalker F1. Even for small farmers aimed to local organic markets they are not suitable. Similar problems but to a lesser extent were found in broccoli, i.e. good development but more heterogeneous head production and shapes. As a result, the tested cell fusion free broccoli accessions were not suitable for big cooperatives aimed to big organic retailers and exports like SURINVER (i.e. conventional producers, evolved partly to the organic markets of Europe, but keeping conventional minds still searching for excellent uniformity). Nevertheless, these cell fusion free broccoli accessions could have an opportunity among small local organic farmers. In any case, new breeding programs integrating local germplasm (more adapted to specific agro-climatic conditions of Spain) are necessary in these crops.

In **Portugal**, with the results of this year, it was possible to observe in a more grounded way the differences between the various varieties tested. In the broccoli crop, a crop with elevated consumption in Portugal, it was clearly observed that the development of open-pollinated varieties was much weaker than F1 hybrids, compensated a little, according to some, with a more interesting flavour. However, the lack of uniformity complicates the production and consumption of open pollinated broccoli varieties for the organic market. Regarding the kohlrabi crop, the results of this year were promising, starting with the overall development of the entire trial. It is very interesting to note that some varieties of open pollination, with emphasis on the Enrico variety, offer productivity comparable to F1. However, this is a crop that, unlike broccoli, is still quite unknown in Portugal by the consumer, and its consumption is quite low. However, due to the greater robustnes in terms of susceptibility to pests and diseases and resistance to adverse pedo-climatic conditions, it can make it, in the short / medium term, as a crop of interest to be grown under organic conditions in Portugal. With this trial we were able to understand which are the best open pollinated varieties adapted to this pedo-climatic conditions choosing for next year only the most interesting ones in comparison with F1.

The results of the **Italian** trials (kohlrabi, broccoli) were consistent with those from Spain and Portugal. In Italy too, it was shown that organic open pollinated kohlrabi varieties are available that can compete with commercial F1 varieties. Enrico, but also Fridolin, Sako or Sat79 can be recommended for value chains that distinguish non only cell fusion free cultivars but also cultivars of organic breeding initiatives. Regarding broccoli, the differences between organic open pollinated varieties and commercial F1 were much larger in Italy too. Rasmus showed to be the best OP candidate. However, it was still characterised by a larger harvest window and a greater visual heterogeneity.

The main results of **French** trials were similar to those from Spain, Italy and Portugal for kohlrabi. As this particular species has no commercial standard type, heterogeneity of aspect or differences of aspect between two cultivars are not a problem for direct marketing, and for long chain retailing either, so that some of the OP varieties and genetic resources evaluated can compete with F1 commercial varieties (Sat 21 – Enrico, Fridolin, KS-KOK-77-NOR) in terms of aspect and yield. No long-term breeding work is needed to develop new OP varieties from these genetic resources (like BRA 2151 A and BRA 1889 for violet type, or BRA 2086 or BRA 1475for green type). Regarding broccoli, Britain farmers have to deal with retailers needs and requirements in terms of quality and homogeneity, so

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no OP variety (visual heterogeneity, larger harvest window, lower quality than F1 hybrids, and lower yield) has a sufficient quality to meet the French producers and market needs.
From a general point of view, one must note that, in all trials, a strong emphasis was put on testing open pollinated varieties originating from organic breeding initiatives and not only cell fusion free varieties. Indeed, according to the positive list of cell fusion free cultivars (BNN, 2019), there are at the moment many more cell fusion free F1 varieties that might have a higher potential than the tested OP varieties. Depending on the needs of the value chain, it might be interesting to test more cell fusion free F1 varieties based on self-incompatibility, especially in those crops (broccoli, cauliflower) where there are almost no interesting OP varieties adapted to Southern European conditions.
Regarding the most challenging Brassica crops, broccoli and cauliflower, it must be noted that specific adaptation seems more important than e.g. for kohlrabi. Indeed, cauliflower varieties are strongly segmented and are often specifically recommended for each cultivation area and time. It is therefore not surprising that organic bred OP varieties that were selected for Central European summer or autumn cultivation did not perform well under Southern European winter/spring conditions. In order to close the winter gaps in Southern Europe with cell fusion free cultivars, breeding work for local adaptation would be necessary in those areas. This is one of the most important findings of LIVESEED as generally the available vegetable varieties, in contrast to cereal varieties, are grown on a global scale.
Moreover, LIVESEED contributed in a joint effort with several organic farmers association to publish a positive list of cell fusion free Brassica vegetable for Central Europe and another one for Southern Europe is under process. This is very important for farmers and retailers alike, as certain private labels have banned the use of cell fusion derived CMS hybrids. To collect such information is very time consuming and requires interviews, expensive tests for cell fusion and/or evaluation of pollen fertility. Therefore, ECO-PB is promoting that breeding techniques should be disclosed when applying for variety registration, so that the list of cell fusion free Brassica vegetables and other crops could eventually be provided on request by the Common Plant Office (CPVO).

4.3. Apple

The main objective of LIVESEED Task 3.4.4 on apple breeding network is to improve breeding for organic apple production through a network that will join forces, share knowledge and genetic resources. Besides networking, the LIVESEED apple task involves test cultivation on 5 organic farms in different growing regions of Europe in preparation for the market introduction of candidate cultivars from biodynamic breeding Poma Culta. This activity started in 2017 and the pilot cultivation were regularly visited and assessed. Each tree was entered into the barcode-based computer system of the Poma Culta (Breeders Database) for annual assessment. First apple fruits are expected in 2020. FiBL-CH supported the Poma Culta apple breeding with respect to resistance breeding for new diseases, implementation of molecular markers for parental selection and sensory testing of candidate cultivars from Swiss nursery.
Following key factors were identified in order to improve efficiency of organic fruit breeding and cultivation:

- Market introduction of organic cultivars (awareness raising of added value of pesticide free production, negotiation with traders to promote new cultivars)
- Addressing farmers, traders and consumers needs
- Common database
- Knowledge sharing (e.g., videos on practical breeding steps etc.)

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Compared to annual crops the market introduction of new apple cultivars is much more difficult, as the nursery usually has a lifetime of 15 to 20 years. Thus, planting new cultivars is a considerable and risky investment for organic growers as the do not know how the cultivar will perform under their conditions and if the traders and consumers will accept the fruits of new cultivars. It is challenging to introduce and promote new apple cultivars in the organic markets as consumers are rather conservative in purchasing know cultivars and expect to obtain same cultivars from both conventional and organic production. For example the apple cultivar 'Gala' is highly demanded by organic traders although the cultivar is very sensitive to several pest and diseases. Therefore, awareness raising for the added value of the benefit of new organic apple cultivars along the whole value chain is needed.
Establishing a trustful network between different actors involved in organic apple breeding, cultivar testing and cultivation across Europe allows for (i) faster exchange and test cultivation opportunities to obtain reliable data on crop performance, (ii) common promotion strategy for market introduction from farmer, trader to end users, and (iii) recommendation on legal framework to ease the registration on new organic apple cultivars as foreseen in the upcoming temporary experiement on organic varieties suited for organic production (new organic regulation EU 848/2018).
The necessary re-orientation of the apple breeding from an organic point of view and requirements of varieties for organic cultivations were pointed out by FiBL-CH. Gaps in the current organic assortment and new recommended apple varieties and their advantages were also discussed in BioFach 2019 LIVESEED Apple task workshop. Information exchanging would involve testing protocols/methodology and a list of prebreeding material with relevant testing results. The network would create incentives via material exchange and promote common approach on breeding (common database, open source seed as common good). In order to tackle confidential constrains in material exchange and knowledge gained, trust and time are needed, as well as information about patents and also cross action. In order to meet farmers needs the network will try to promote participatory activities accompanied by consulting issues. Promoting breeding to end users and public would mean the use of participatory approaches and requires a strong consortium and a task force to boost organic breeding and varieties (LIVESEED and relevant projects). There is a need, also, in developing positive arguments like reduced pesticide application, copper-free organic production, improved/diversified taste, or saving biodiversity when commercializing or advertising organic bred apples. The association bioverita developed protected label to distinguish products derived from organic plant breeding along the whole value chain. The bioverita labels also guarantees organic consumers that the used breeding techniques are compatible with organic principles and no genetic engineering techniques or cell fusion was involved. A booklet with different approaches and experiences translated in different languages and relevant videos to be created for this purpose would also be very helpful.
The ongoing initiative of organic apple breeding thematic network under LIVESEED project on collaboration and material exchange was discussed in a LIVESEED workshop during the 19thEcofruit conference (Germany, February 2020). The discussion focused on elaborating a European Cooperative Participatory Network of Organic Fruit Breeding. This would need a broad platform of shared information and material exchange connecting different ongoing and existed projects.
Information should include:

- testing protocols/methodology
- prebreeding material list (e.g. parental lines)
- candidate selections with relevant testing results

Information data should be customized in to open, easily and free accessible form. A permanent institution was suggested to undertake the organization and creation of such a database collaborative

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and platform information exchange system. Organizing responsibility, also, should be shared among existing institutions and people involved. Database could enriched by and integrate information from existed networks or institutions (e.g. European cooperative Programme for Plant Genetic Resources, ERCPGR).

4.4. Winter wheat

In the Task 3.4.4 on supporting organic winter wheat breeders by providing modern breeding tools to improve resistance to seed borne disease common bunt caused by *Tilletia caries* the focus is on combining competences to develop common tools. Like in Task 3.4.1 the different tasks are shared between partners: wheat phenotyping (AGROL), genotyping and analysis (UNI KASSEL), validation of results (AGROL, Dottenfelder Hof, Cultivari). By May 2020 the genotyping and phenotyping of 270 lines with 10 different virulence strains of common bunt have been completed and statistical analysis of genome wide association studies (GWAS) will be conducted in the last year to identify markers linked to at least 7 new resistance sources. Moreover, the respective LIVESEED partners are in exchange with the sister project ECOBREED to use more synergies. While LIVESEED is identifying markers for new sources of commen bunt, ECOBREED is applying already published markers. Together both projects will result in a tool box of 10 resistance sources for more efficient breeding for bunt tolerance. In combination with LIVESEED actitivies in WP2.3.2 on seed health strategies for common bunt including seed treatments this will considerably improve the availability of high quality seed of suited wheat cultivars for organic and low-input farming.

Another task was also to build new networks between practical organic breeders and researchers involved in molecular plant breeding. The involved breeders have large knowledge and several decades of expertise on organic winter wheat breeding and bunt resistance breeding, this the university partner is specialized on molecular markes. Some of the involved partners had already good collaboration (although also some competition), they share same vision and background. Their trustful relationship eases exchange of knowledge and material: they test each other's material and they use it for further breeding. Extending the network by involving new partners needs more time for discussion to reach common understanding and fruitful collaboration. This is especially true for actors from different disciplines but also if new staff is engaged in the middle of the project. In order to strengthen the collaboration it is indispensable to have frequent exchange between breeders and researchers in order to agree on research goals, genetic material to be tested, trial set up, interpretation of results, etc. It is important to align the timelines of molecular marker results with the selection deadlines of breeders. The cooperation needs to fulfil the expectation of the different actors, i.e. on one side scientific innovations to be published in scientific journals and on the other side concrete tools that can be used by breeders for marker assisted selection (MAS) in collaboration with contract service providers. In this respect the cooperation between LIVESEED partners who will identify markers linked to novel bunt resistance sources and ECOBREED partners (BOKU) who are focusing on MAS using already existing markers is of great advantage for both. ECOBREED and LIVESEED will organize together an international workshop on bunt and smut diseases in November 2020 in Austria. This will not only allow dissemination of results but also to strengthen and enlarger the networks of organic plant breeders with respective researchers dedicated to topics relevant for organic agriculture. It is also planned to apply for a respective COST action to institutionalizing such networks and cooperation on EU level.

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4.5. Tomato

In task 3.4.5 on supporting participatory tomato breeding in Italy and Spain focused on different PPB approaches for local adaptation considering the different different socio-economic context.

Tomato has many advantages to face PPB initiatives: i) very much studied crop, ii) many methods available for breeding, iii) relatively easy to find small organic initiatives or encourage new ones to engage in PPB as it is a very important and attractive crop, iv) a plethora of landraces and diverse populations is available in most countries, v) easy reproduction (annual crop, autogamous, just a few plants necessary for seed multiplication), vi) an important part of consumers are willing to discover/recover new tastes and flavors.

As disadvantages: i) still F1 modern varieties grafted on rootstocks are dominate the market, even in organic, ii) a strong effort is needed to make consumers aware of the opportunities of landraces for organic (part of diffusion in our open days), iii) organic tomatoes may reach too high prices and many consumers have the idea that they are only for rich people.

After two years of evaluations and PPB including farmers, technicians and general public we can say that the landraces have a good potential for the organic sector. In our trials many accessions have showed a very good performance under organic open field conditions. Also, the preferences from organic local consumers in Spain (both Valencia and Southern Spain-Andalusia) are high if prices are not excessive. In fact, good organic tomatoes from Negro Segureño can be bought at 2.5 €/kg in local markets of Andalusia or Valencia. Also, there is an interesting opportunity for mixtures of different varieties (basket mixtures) in the frame of organic market. This is very good for diversification of production and contribute to maintain agrobiodiversity.

Many small-medium farmers are willing to develop their own varieties, so they offer in-kind collaboration for small initiatives and to become farmers-breeders, but not more than 100-300 plants for evaluations, depending on the farmer. Then it is relatively easy to find collaborations with farmers. Nevertheless, some competition can be found among close local farmers, so supporting/facilitating breeders (like UPV) must be careful to avoid some personal problems. In kind contribution for bigger scale trials (1000-2000 plants, like main trials in Valencia and Cadiz) are more difficult. Even if medium-size or big-size cooperatives are involved they require some compensation as this is an intensive crop in terms of management and costs, in comparison to others like Brassica, wheat or lupins.

Very similar concerns and opportunities have been found in Italy by RSR and their associated partners linked to participatory breeding activities with CCP tomatoes.

The PPB program 3 coordinated by CREA during the two years experiment, developed genotypes with a good level of homogeneity and adaptation to organic farming under different pedoclimatic conditions in Italy. Furthermore, the PPB allowed to select plants showing important agronomic traits useful for farmers needs and to respond to local market requests. Farmers were very interested in our trials by giving helpful aid and collaboration. Unfortunately, the trial conducted in the North (Padova) failed due to the adverse environmental condition which should be always take into consideration during open field cultivation.

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5. Key factors for strengthening small breeding initiatives

5.1. Lessions learned

Based on the knowledge exchange between the partners of the five different breeding networks it was possible to deduce a number of key factors which are important to strengthen small breeding initiatives.

The five breeding networks focusing on different challenges related to specific crops had various aspects in common, but also contrasted clearly in other aspects. First of all the status quo of existing organic breeding activities is very different. For major crops like wheat and tomato there are several organic breeding initiative, while for Brassica vegetables or fruit breeding there are less activity. This was also supported by the EU wide survey on organic plant breeding initiatives, of which 31 were engaged in vegetables, 28 in cereals, 17 in legumes and only 10 in fruits (see Deliverable D3.9.). Especially fruit breeding is hampered by the long duration of the breeding process (>20 years). Similarly, the knowledge on genetics and breeding tools (e.g. molecular markers) is much more advanced in wheat, tomato, or apple, compared to Brassica vegetables or lupins, which has received little attention in plant breeding in Europe. Hence making progress in lupin breeding is not that easy as the toolbox is not that big yet compared to tomato and wheat. Although Europe is quite strong in vegetable breeding there are quite different breeding approaches between the conventional and organic sector. While for organic breeders targeting cell-fusion free vegetables demanded by the traders in Central Europe (BNN), most research organisations and commercial breeding companies focus exclusively on cell fusion derived germplasm with cytoplasmatic male sterility (CMS) for efficient F1-hybrid breeding. This limits not only the cultivar choice of organic farmers but also strongly prevents that such breeding material can be used in organic breeding. Thus, organic breeders cannot profit from the general breeding progress but need to develop own germplasm pools.

While for the organic lupin, Brassica and apple breeding it was most important to link actors across Europe to reach critical mass to enhance breeding progress, for wheat and tomato it was more important to start regional networks. One major aim was also extend the organic testing network in different agro-climatic regions and to link different competences. This is especially true for the application of molecular markers in organic breeding which was main focus of the wheat network and to a certain extend for the lupin, tomato and apple network. In the Brassica network markers are applied to identify cell fusion free cultivars for the established positive cultivar list. In each network practical breeders and research institutions worked together to close the most urgent breeding gaps using diverse tools and approaches depending on species and the already existing breeding initiatives (*Table 4*).

Of key importance is that the network member share a common vision of the goal and agree how to reach that goal. There was also a common understanding among LIVESEED partners to focus on applied research and cultivar development and that networks are open to other stakeholders. Partners from different background needed more time to reach to that stage.

Of the five case studies, the lupin case where there are great breeding gaps has profited most from the joint collaboration and outreach to other partners outside LIVESEED has already started.

Due to the trustful collaboration and developed population and breeding material there is high potential for improved cultivars. Also the tomato and wheat case seems to have most potential to further continue after the project. There the collaboration with BRESOV and ECOBREED are of great interest. Thanks to the strong engagement of the facilitator to establish a new EU wide organic fruit breeding platform there is a strong will for collaboration.

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Table 4. Synopsis of main characteristics of the five crops taken into account in the five case studies

Crop	Involved people	earlier collaborations	Role of facilitator	testing each others material	common methods available	crop type	available varieties	knowledge on genetics	Take into account the market	Main breeding gap
Lupin	bringing competences together (5 partners)	few	Functioned well: stimulated collaboration with other partners	yes	Tools are developed and shared	annual / grain legume / mainly self pollinating	limited	limited	partly	Many challenges as little breeding and research was done, pre-breeding stage
Brassica	share common goal and vision, common action needed to be developed	yes, partly	4 times changes in personnel hampered facilitation	yes	available, developed together	biannual / vegetable / outcrossing	rather limited	available, although breeding methods for OP population enhancement are lacking	Yes	Cell fusion free varieties for Southern Europe Working with the value chain
Apple	share common goal, vision, action	some	Very strong, was able to build network outside of LIVESEED partners	not yet	Common database (to be developed)	perennial / fruit / outcrossing	rather limited	Available, limited knowledge on horizontal resistance	Yes	Robustness against many pest and diseases and high fruit quality, On-farm testing of candidate cultivars
Wheat	bringing competences together	some	Facilitator was not so active	yes	available	annual / cereal / self pollinating	many	thoroughly studied	No	Organic varieties with good resistance to common bunt
Tomato	share common goal, vision and common action	within country	Functioned well: stimulated good collaboration	No, as goal was on local cultivars	available	annual / vegetable / self pollinating	many	thoroughly studied	Yes	Collaboration with the value chain and farmers Development of local varieties with good flavour and taste

5.2. Future perspectives

It is anticipated that the trustful relationship that has been build up among the actors will lead to a continuation of the breeding network beyond LIVESEED in order to tackle future challenges. Therefore, further breeding gaps were identified in close exchange with related projects BRESOV (tomato, broccoli, snap bean) and ECOBREED (wheat, soybean, potato, buckwheat) conducting intensive research on organic plant breeding. Research gaps for the different crops are listed below:

Lupin

- Application of screening tools for anthracnose tolerance in segregating populations
- Validation of identified QTL and their potential for marker assisted selection for anthracnose resistance, ph-tolerance, drought in segregating populations
- Utilize created sweet CCP populations for further breeding and evolutionary populations to local conditions in Europe
- Development of selection tool for low alkaloid content allowing for high throughput screening (molecular/photometric)
- Explore new sources for low alkaloid content
- Link to value chain partners of the food industry interested in innovative plant based protein products
- Research on breeding for weed competition and intercropping or undersowing crops

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Brassica

- Initiation of new breeding initiatives for cell fusion free cultivars in Southern Europe
- Research of different breeding methods for recurrent population enhancement to develop cell fusion free OP varieties especially for broccoli and cauliflower to reach homogeneous produce
- Development of markers for self-incompatibility genes to explore possibility of cell fusion free SI hybrids or populations
- Join projects with commercial companies still multiplying SI hybrids for the organic sector (Bejo, Tozer seeds)
- Intensify collaboration among actors involved in Brassica breeding for the organic sector
- More exchange with traders / consumers to compromise their high expectations on homogeneous produce appearance for the sake of better taste but less homogeneous cell fusion free OP cultivars
- Research on new emerging diseases, pest, drought, heat, taste, storability, nutritional components (see BRESOV)

Apple

- Implement European Cooperative Participatory Network of Organic Fruit Breeding
- Extend test cultivation at organic farms of candidate cultivars
- Implement common database
- Explore opportunities that allow for faster and more cost-efficient variety release of apple
- Research on new emerging pest and diseases and development of cost efficient screening tools
- Research on improved taste and low allergy content, good storability
- Establish organic breeding in other fruit crops like pear, peach, apricot, cherry and berries

Wheat

- Conversion of identified QTL for bunt resistance in cost efficient PCR markers
- Combined toolbox for bunt resistance gene screening of LIVESEED and ECOBREED
- Pyramidizing resistance genes in inbred lines, line mixtures and CCP populations
- Research on new emerging diseases, pest, drought and heat and flooding tolerance (see ECOBREED)
- Research on good baking quality with medium protein content and high nitrogen use efficiency
- Research on non-coeliac sensibility of wheat and methods to select for better tolerance of those customers

Tomato

- Extend participatory breeding approaches to other regions and crops in Europe
- Research on large number of diseases, pest tolerance, water and nutrient efficiency, low temperature greenhouse, taste and storability (see BRESOV)
- Research on tomato rootstocks specifically suited for organic production and plant soil microbe interaction

5.1. Transfer to initiate and strengthen small scale breeding initiatives in other crops

As the five case studies cover a large range of annual, perennial, self-pollinating and outcrossing species as well as different goals and approaches they can serve as blueprint to transfer to other crops and agro-climatic regions. Several factors were identified among the LIVESEED partners of the respective case studies that are important for successful strengthening of small organic breeding initiatives by collaboration. Before starting new initatives and networks for other crops there should be thorough analysis to define market demand and existing gaps and map how the case study is embedded in the overall framework with respect to science and technology, market, society, norms and regulations (*Figure 1*). *Table 4* could be used as template to get a first overview. Key factors for collaboration between breeding projects/initiatives depending on

Crop species:

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- Crop type (reproduction system, annual/bi-annual crop, rate of reproduction)
- Crop specific related knowledge: the current status of knowledge on breeding
- Breeding approach (focused on homogeneous varieties, populations, etc)
- Type of material tested (population, breeding lines, varieties, etc)
- Possibility of combining / integrating / connecting different approaches
- Availability and access of methods and infrastructure for testing (visual, lab analysis, etc)
- Common Evaluation: set up of design, level of participation, sharing of templates
- Access and exchange of material (limited, free, etc)
- Scale / economic importance of the crop
- Timeline (congruency in time of activities)

Actors and stakeholders:

- Identification of actors with common interest / common need and complementary competences
- Engaged and moderating facilitator to initiate and maintain collaboration
- Common definition of goal /focus (variety improvement / specific traits)
- Building trust among partners via frequent exchange
  - Time and intensity of former collaborations
  - Type of actors involved (level of congruency between skills and knowledge)
  - Group size
  - Diversity in backgrounds (researcher, breeder, farmer, value chain)
  - Type of organisation (non-profit, public, commercial)
- Development of research plan / distribution of tasks and responsibilities / timelines
- Agreement on IP rights
- Regular exchange of knowledge among partners and update of research plan
- Communication to reach other stakeholders
- Engagement of farmers / value chain / consumers / society

Economic, regulatory and societal framework in respective countries

- Market demand by organic and low input farmers, processors, traders, consumers
- Regulatory limitations for marketing organic varieties (now new scope for organic heterogeneous material / planned temporary experiment for organic varieties suitable for organic agriculture as mentioned in the new EU organic regulation 848/2018)
- Political support and public recognition for added value of organic agriculture and plant breeding
- Organisation of

Funding resources

- Timely limited project based funding
- Charity foundations
- Funding via commercial seed sale or licenses/breeding contribution on seed
- Funding via farmers associations
- Funding via downstream value chain partners

There are several technical or operational factors that depend on the **crop species and breeding gaps** to be tackled as described above. In the best case the collaboration will result in common toolbox of approaches, methods, improved breeding material, extended organic testing networks and eventually improved organic cultivars for farmers.

In addition to the technical toolbox, so-called soft skills will be crucial to determine the success of a collaboration among **actors and stakeholders**. Across all the case studies the trustful collaboration among actors are key. First we need to identify relevant actors that have common interests or needs and a facilitator who is initiating the process. It is of course much easier to agree upon common goals and approaches if there is a small group, which had already collaborated before and have similar background. Experience from the former project DIVERSIFOOD also highlights that endurance is and great amount of flexibility is needed to adapt to changing conditions and interests of different actors. Therefore, multi-actor participatory approaches with joint decision power cannot be planned as

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streamlined as top down projects but needs continuous discussion and adjustments to reach the common goal. If partners are not sufficiently involved there is high risk of drop out of partners if the project ends. Therefore, the role of a competent and **engaged facilitator** cannot be overestimated.
It is important that the goal of the breeding network meets the **demand of the market**. Therefore, it is important to integrate value chain partners and engage them as early as possible. This involves not only farmers who buy the seed, but also food processors, traders and consumers.
The overall goal of LIVESEED on boosting organic seed and organic plant breeding will need support from the policy makers and society. With the advent of the **new organic regulation EU 848/2018** the actual availability new cultivars for organic farmers will be much improved. Due to the simple notification procedure foreseen for the marketing of organic heterogeneous material (OHM) the CCPs and the farmers selected landraces of tomato can be commercialized. This is also of importance for the CCPs of lupin, CCPs of wheat with bunt genes, or Brassica which is as outcrossing species very heterogeneous unless hybrids are developed. Moreover, the foreseen temporary experiment on easier market access of organic varieties suited for organic production via adjusted variety registration (DUS and VCU) will be important for all crops but maybe specifically for apple, where the breeding and official DUS testing requires lots of time and resources. Therefore, LIVESEED was very engaged and in fruitful exchange with respective policy makers (Task 2.1.2 and 2.1.3). Communication and awareness raising of the added value of organic agriculture and organic breeding is indispensable to reach consumers and society. **Society** will eventually decide about the sustainability of our future food production. Organic breeding can contribute considerably to the "farm to fork" strategy of the EU commission published in 2020.
Most limiting factor of small breeding initiatives including the actors of the five case studies is the insufficient **financing**. This was already stressed in the EU project DIVERSIFOOD and confirmed in the breeder and seed supplier survey in Task 1.1. (Deliverable 1.3). As organic agriculture aims for many different crops species and large choice of cultivars per crop the revenue of organic seed sale per cultivar will not be able to cover the running costs of organic breeding programs. Presently, most private breeding initiatives relay on insecure and mixed funding of timely limited research funds, own resources, charity foundations, contribution of farmers associations and in view cases by contribution of value chain partners. To tackle the issue of insufficient financing several workshops have been conducted in WP3 (see Milestone 3.5) and WP4 on developing and modelling business models for organic seed and breeding. Alternative financing models have been developed aiming for pool funding of all value chain partners to join forces to co-invest in organic plant breeding to secure the integrity of their supply (Winter et al. 2020 submitted). The perspective of long-term funding is most decisive for (i) the future of existing organic breeding initiatives to attract young breeders, (ii) entrepreneurs who want to start new organic breeding programs, and (iii) for conventional seed companies to invest in organic seed and breeding. The economic aspects and business models will be analysed in more detail in WP4 and described in upcoming deliverable 4.3 (Report on analysing and optimising organic seed markets, supply chains and business models of breeders and seed producers).

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